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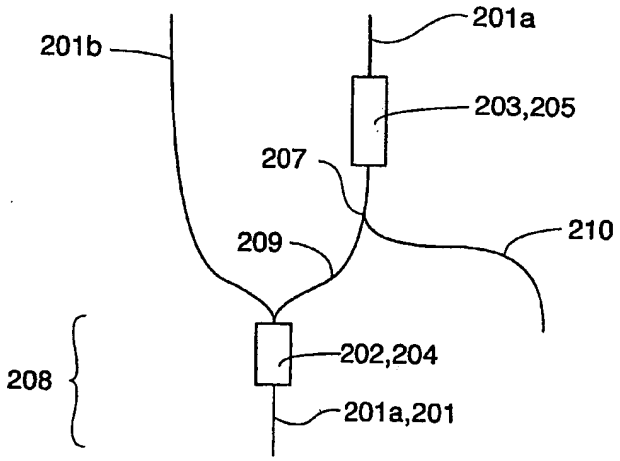
(54) Title: FLOW PATHS COMPRISING ONE OR TWO POROUS BEDS



(57) Abstract: A microfluidic device that comprises a microchannel structure in which there are one, two or more flow paths (101;202a,b;302a,a',b) all of which comprises a porous bed I (104,204,304) that is common for all of the flow paths and exposes an immobilized reactant R that is capable of interacting with a solute S that passes through the bed. The characteristics are that at least one of the flow paths comprises/comprise a second porous bed II (105,205,305) that is placed upstream to porous bed I (104,204,304) and is dummy with respect to interaction with solute S but capable of interacting with a substance DS that is present in a liquid aliquot together with solute S and is capable of disturbing the result of the interaction between solute S and said immobilized reactant R. There is also disclosed a method utilizing the device and variant of the device in which the immobilized R is replaced with a generic affinity ligand L_I, and/or porous bed II exposes a generic ligand L_{II} that may be different from L_I.

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FLOW PATHS COMPRISING ONE OR TWO POROUS BEDS.

TECHNICAL FIELD

The invention relates to a microfluidic device and a microfluidic process comprising steps that are carried out in a particular kind of flow path(s) **(101;201a,b;301a,a',b)** of a microchannel structure of the device. The part of the process comprises that a solute S is allowed to interact with a reactant R that is immobilized to a solid phase material. The particular flow path(s) concerned comprises/comprise the solid phase material in the form of a porous bed I **(204,204,304)** in the flow path(s) **(101;201a,b;301a,a',b)**.

The process comprises for instance:

a) removal including separation of solute S from the liquid, i.e. solute S is retained or degraded by the solid phase, e.g. by affinity to groups on porous bed I, and/or
b) a catalytic reaction, e.g. an enzymatic reaction, with one component of the catalytic system used being the immobilized reactant R, and/or
c) solid phase synthesis.

For variants (a) and (b) the process of the invention is typically part of an analytical assay or protocol in which an uncharacterized aspect of a reactant (= analyte) is characterized/determined, e.g. identity, structural features, amount in absolute or relative terms such as concentration, etc. The term "reactant" comprises analytes and reagents.

The term "solute" refers to a substance that is in truly dissolved form or in suspended form including colloidal form. The term thus includes microorganisms such as bacteria, mould, viruses, bacteriophages etc, and fragments thereof when they are used in suspended forms.

All patents and patent application cited herein are incorporated in their entirety by reference.

BACKGROUND PUBLICATIONS

WO 02075312 (Gyros AB) describes a microfluidic device in which there is a microchannel structure which each comprises a separation unit for removing particulate material from a liquid aliquot. The liquid aliquot contains also a solute that in a subsequent step is a reactant in an affinity based assay.

PCT/SE2004/001424, WO 0147638 (Gyros AB), WO 03098302 (Gyros AB), WO 02075775 (Gyros AB), WO 02075775 (Gyros AB) describes various structures for which it has been suggested with upstream processing of a liquid sample followed by downstream processing of the result of the upstream processing, possibly including a determination step.

US 6,632,655 (Caliper), Piyasena et al (Anal. Chem. 76 (2004) 6266-6273) and Buranda et al (Anal. Chem. 74 (2004) 1149-1156) describe a porous bed that comprises segments. The bed is used in multi-analyte assays.

PROBLEMS THE INVENTION AIMS AT SOLVING

Many samples, such as biological fluid samples, many times contain disturbing substances that are capable of negatively affecting results of reactions between a solute and a reactant immobilized to a porous bed. This has created problems for us in sandwich assays as outlined in WO 02075312 (Gyros AB) and WO 04083108 (Gyros AB), i.e. with the analyte being equal to solute S above. If a substance is disturbing or not or to what degree will depend on kind of sample, among others. A disturbing substance may be a dissolved compound, an aggregate and/or a particulate material including also various kinds of mal-functioning reagents (see below). For biologically derived samples particulate material may be cell debris and the like, lipids etc. The problem encountered may be linked to type of reactants, e.g. analyte. Membrane associated biological analytes are often accompanied by relatively large amounts of particulate material disturbing an assay. Samples from cells, tissue and body fluids are typically difficult to handle in microfluidic devices. There may be heterophilic antibodies that interact with antibody reagents in an undesired manner in immune assays. Reagent compositions may contain forms that disturb the result of an assay, for instance by creating signal responses that are comparable to or higher than normal back ground responses. Labelled reactants may contain forms that have an abnormal density of labelled groups thereby differing sizely and/or chemically from the normally labelled forms.

OBJECTS OF THE INVENTION

The objects of the invention are to provide improvements applicable to microfluidic devices in relation to the above-mentioned problems as well as to other problems. This means methods and microfluidic devices enabling improved detection limits, analyte specificity, diagnostic sensitivity and specificity, precision, dynamic range, recovery etc in analytical assays in which a characteristics of an analyte is determined. The objects of the invention thus

aim at a) limits of detection for analytes $\leq 10^{-6}$ M, such as $\leq 10^{-9}$ M or $\leq 10^{-12}$ M or $\leq 10^{-13}$ M or $\leq 10^{-14}$ M or $\leq 10^{-15}$ M or $\leq 10^{-16}$ M, b) dynamic ranges that are two, three, four, five or more orders of magnitude (M), c) precisions (CV) within ± 20 %, such as within ± 10 % or within ± 5% or within ± 3%, d) recoveries ≥ 70 % such as ≥ 80 % or ≥ 90 % or ≥ 95 % or around 100 % or more. For other process protocols such as of the type indicated under the heading "Technical Field", the objects relate to improved performance with respect to other relevant variables, e.g. number of acceptable experiments/runs of a process per time unit, precision, reproducibility in yield etc.

FIGURES

Figures 1a-b illustrate two variants having two different porous beds linked in series in a common flow path.

Figure 2 illustrates a variant with two flow paths that have a common porous bed. One of the flow paths has a second porous bed upstream of the common porous bed.

Figure 3 illustrates a variant with three flow paths that have a common porous bed and one additional porous bed in two out of the three flow paths.

Figure 4 illustrates results of experiment 2A.

Figures 5a-b illustrate results of experiment 2B

INVENTION

We have realized that these objects can be complied with by

a) providing a solid phase material in the form of a porous bed II **(105,205,305)** in a flow path **(101,201a,301a,a')** of a microchannel structure in a position upstream of porous bed I **(104,204,304)** where porous bed II **(105,205,305)** is dummy in relation to solute S but not in relation to the disturbing substances, and

b) transporting solute S dissolved in a liquid aliquot containing one or more disturbing substances through porous bed II **(105,205,305)** before the solute and accompanying liquid is transported through porous bed I **(104,204,304)**.

Porous bed II **(105,205,305)** is dummy in relation to solute S, i.e. solute S is able to pass through the bed unaffected while disturbing substances are neutralized in the bed by interaction with the solid phase material. Neutralization means degraded, captured or otherwise hindered from passing into porous bed I **(104,204,304)** simultaneously or before solute S. Solute S will thus be allowed to interact with the solid phase material of porous bed I

(104,204,304) in the absence of the disturbing substances that have been neutralized in porous bed II (105,205,305).

The first aspect of the invention is a microfluidic device that comprises one, two or more microchannel structures. Each of the microchannel structures comprises one or more flow paths (101;201a,b;301a,a',b) that comprise a common reaction microcavity I (102,202,302) that retains a solid phase material in the form of a common porous bed I (104,204,304). This bed exposes or is intended to expose an immobilized reactant R that is capable of interacting with a solute S that passes through the bed. Common in this context means that all the flow paths pass through the this reaction microcavity I/porous bed I (102,202,302/104,204,304).

The main characteristic feature of the device is that the microchannel structure comprises a second porous bed II (105,205,305) that is placed upstream of porous bed I (104,204,304). Porous bed II (105,205,305) is placed in reaction microcavity II (103,203,303) that may be physically separated from reaction microcavity I (102,202,302) or joined with reaction microcavity II (103,203,303) to form an enlarged reaction microcavity I + II. If the two porous beds I and II are placed in such a reaction microcavity they form a joint porous bed comprising an upstream segment corresponding to porous bed II (105,205,305) and a downstream segment corresponding to porous bed I (104,204,304). In other words the downstream end of the upstream segment is abutted to the upstream end of the downstreram segment. This includes that a porous membrane (106) may be placed between the segments in order to secure that the two solid phase materials are not intermixed at their interface. Porous bed II (105,205,305) is dummy with respect to interaction with solute S but not with respect to disturbing substances.

A microcavity, microconduit, or other functional unit is said to be in liquid communicting with another microcavity, microconduit, or other functional unit in the case liquid is intended to be transported from the former to the latter.

Disturbing substances

A disturbing substance is capable of disturbing the outcome of one or more of the desired interactions taking place in and downstream of porous bed I (104,204,304) in the sense that the substance has a negative impact on the result obtained. For processes in general there are a number of quality parameters (results) that may be adversely affected, e.g. amount of time

required for performing a single experiment or run of the same process/method (increasing), productivity measured as number of runs per time unit (decreasing), recovery or loss of one or more of the individual reactants used (decreasing or increasing, respectively), yield of one or more products obtained (decreasing), precision related to product parameters such as yield, purity, etc (increasing), For analytical assay protocols one or more of the following quality parameters may be adversely affected: precision (lowering), limit of detection (increasing), dynamic range (narrowing), analyte sensitivity (lowering), diagnostic sensitivity and specificity (lowering), recovery (lowering), undesired loss of a reactant used (increased), such as of the analyte etc.

A disturbing substance may be in dissolved form and/or in particulate form. Particulate forms include any form that is not a dissolved form. In particulate forms a disturbing substance may exert its adverse effect by clogging porous bed I or other porous beds that may be present downstream of porous bed I. Truly dissolved forms of a disturbing substance have a low risk to clog a porous bed, unless they easily are transformed to particulate forms upstream of or in a bed during the process. Dissolved forms of disturbing substances may exert their disturbing effects:

a) by inherently comprising measurable characteristics that coincide with or otherwise disturb the characteristics utilized for the measurement of the desired product or by participating in reactions that during the process result in an entity comprising such measurable characteristics, and/or

b) by interfering with the desired reactions of the process, for instance by neutralizing or otherwise consuming the activity of added reactants (e.g. the analyte and/or one or more of the reagents added), and/or

c) by interacting with inner surfaces of the microchannel structure used thereby causing enhanced undesired interaction of one or more of the reactants with inner surfaces (e.g. undesired binding to solid phase material and/or to inner surfaces), and/or

d) by transformation to particulate forms thereby causing precipitates including deposits and/or sediments in the microchannel structure (e.g. in the porous bed, the measuring zone and/or elsewhere).

In the context of the invention disturbing substances are primarily considered in liquids in which solute S is a reactant, e.g. an analyte or a reagent. Liquids in this context refer to liquids that are intended to be dispensed unprocessed to the microfluidic device.

Reagent-containing liquids: This kind of liquids is typically man-made. Disturbing substances deriving from solvent and buffer components are thus relatively easy to avoid. The reagent part may be more complicated. An organic reagent, such as a bio-organic reagent, is typically manufactured from more complex materials and often contains disturbing substances (contaminants) that are difficult to remove. There is thus a risk that these substances remain in the liquid to be used. Reagents that have been obtained by derivatization of bio-organic compounds, e.g. exhibiting peptide/amino acid, nucleotide, carbohydrate, and/or lipid structure (including steroid structure), may thus be contaminated with starting material, side products etc. In addition to the reagents used for labelling, a labelled reagent may contain a spectrum of molecular entities that differ with respect to activity and/or to number of labels and/or positions at which the individual labels are attached. A conjugate between two substances A and B may contain a spectrum of molecular entities that varies with respect to activity and/or number of A and/or B, ratio between number of A and number of B and/or positions in A that is attached to B and positions in B that is attached to A. Derivatized reagents that have been obtained by fragmentation may contain a spectrum of fragments varying in activity. Reagents in un-derivatized forms may also contain disturbing contaminants, e.g. if they derive from biological material and/or exhibit at least one structure selected amongst peptide or amino acid, nucleotide, carbohydrate, and/or lipid structure including steroid structure. Both derivatized reagents and reagents in un-derivatized form may contain aggregated reagents, e.g. having a spectrum of molecular weights. Thus, some of the molecules in a reagent composition may increase the limit of detection and/or the sensitivity by possessing a higher tendency than the others to participate in side reactions, such as unspecific binding to porous bed I (**104,204,304**) and to inner surfaces of the microchannel structure.

Analyte-containing liquids: This kind of liquids is typically represented by samples that have a significant inter-sample variation with respect to the concentration of analyte and other constituents. An analyte-containing liquid sample typically originates from a complex mixture and thus contains a large number of substances that might disturb an assay carried out according to the invention. Analyte-containing samples thus may derive from the process stream of an industrial or laboratory process or method. The process may be a non-biological process or a biological process in the sense that it utilizes at least some kind of biologically derived material that has been obtained by a chemical or biochemical method, for instance

comprising a bio-organic material that exhibits at least one structure selected from peptide or amino acid structure, nucleotide structure, carbohydrate structure and/or lipid structure including hormone structure. An important source of sample is biological fluids (i.e. liquids), such as blood and various fractions thereof such as plasma and serum, lymph, urine, cerebrospinal fluid (CSF), lachrymal fluid, saliva, intestinal fluid, gastric fluid, regurgitated fluid, sweat, cell homogenates, tissue supernatants, artificial biological fluids etc. The term "biological fluid" also contemplates fractions deriving from one or more of these fluids and comprising a bio-organic compound, typically exhibiting any of the biochemical structures given in this specification. Artificial biological fluids contemplate liquids to which one or more bio-organic molecule of the kinds described herein have been added.

There are three main kinds of disturbing substances that may be present in the analyte-containing liquids discussed above (endogenous substances):

A) Particulate materials as described above. For blood derived samples the term particulate material includes blood corpuscles, such as thrombocytes, blood cells, such as erythrocytes and lymph cells, and fragments of these corpuscles, coagulation products such as fibrin, blood clots and fragments thereof, and other blood precipitation products. For biological fluids in general, particulate material may be cell-related and include cells and their fragments, tissue fragment, neutral and/or ionic lipids in un-dissolved form etc.

B) Substances that form precipitates, sediments or deposits as described above.

C) Substances that interact in an undesired manner with a reagent used in the process so that an entity is formed comprising measurable characteristics that are indistinguishable from the characteristics to be measured. The reagent may be the immobilized reagent R, or a reagent in dissolved form, such as reagent in labelled and/or conjugate form. Heterophilic antibodies are important in this context, i.e. sample endogenous antibodies that are reactive with antibody reagents that expose antigenic determinants that are capable of binding to endogenous antibodies of the sample. Endogenous carrier proteins for the analyte may also be important, in particular if the analyte is of low molecular weight (e.g. $\leq$ 10,000 dalton, such as $\leq$ 5,000 daltons) and/or if its free form is to be determined. Other examples are endogenous constituents that have the same measurable characteristics as a label used. An endogenous enzyme may disturb the measurement if the label used on a reactant utilizes the same substrate as the endogenous enzyme, for instance.

D) Bulk protein, such as albumin, gamma-globulin including particularly IgG and IgA, antitrypsin, and haptoglobulin may disturb and therefore worth removing even if they are not acting as a carrier protein according to (C).

Solid phase materials in porous bed I and II.

The solid phase material in porous bed I (**104,204,304**) and porous bed II (**105,205,305**) may be of the same or different kinds. Thus the downstream bed (**104,204,304**) may be a porous monolithic plug and the upstream bed (**105,205,305**) a packed bed of particles, or the other way round, or both beds may be porous monoliths or porous beds of packed particles. The solid phase material in the two beds may also differ with respect to one or more of base material, particle size (and particle size distribution), porosity, coatings, hydrophilicity/hydrophobicity, swellability, elasticity, rigidity etc.

Suitable particles are spherical or spheroid (beaded) or non-spherical. Suitable mean diameters for particles used as solid phase material are typically found in the interval of 1-100 μm with preference for mean diameters that are ≥ 5 μm, such as ≥ 10 μm or ≥ 15 μm and/or ≤ 50 μm. Also smaller particles can be used, for instance with mean diameters down to 0.1 μm. The outlet of a reaction microcavity and the particles used should match each other so that the particles can be retained in the reaction microcavity. Diameters refer to the "hydrodynamic" diameters. Particle preparations may be monodisperse (monosized) or polydispersed (polysized). Particles may be either porous or non-porous. The term monosized/polysized and porous/non/porous have the same meaning as in WO 02075312 (Gyros AB).

The base material of a solid phase may be made of inorganic and/or organic material. Typical inorganic materials comprise glass and typical organic materials comprise organic polymers. Polymeric materials comprise inorganic polymers, such as glass, and organic polymers that may be of synthetic or biological origin (biopolymers). The term biopolymer includes semi-synthetic polymers in which there is a polymer backbone derived from a native biopolymer. Typical synthetic organic polymers are cross-linked and are often obtained by the polymerisation of monomers comprising polymerisable carbon-carbon double bonds. Examples of suitable monomers are hydroxy alkyl acrylates and corresponding methacrylates, acryl amides and methacrylamides, vinyl and styryl ethers, alkene substituted polyhydroxy polymers, styrene, etc. Typical biopolymers may or may not be cross-linked. In most cases they exhibit a carbohydrate structure, e.g. agarose, dextran, starch etc.

The term "hydrophilic" in the context of a porous bed contemplates ability to absorb water when contacted with water. The expression also means that the inner surfaces of the bed that are in contact with a liquid during the absorption shall expose a plurality of polar functional groups which each has a heteroatom selected amongst oxygen and nitrogen, for instance. Appropriate functional groups can be selected amongst hydroxy groups, ethylene oxide groups ($-X-[-CH_2CH_2O-]_n$ where n is an integer > 1 and X is nitrogen or oxygen), amino groups, amide groups, ester groups, carboxy groups, sulphone groups etc, with preference for those groups that are essentially uncharged independent of pH, for instance within the interval of 2-12. For solid phase materials in particle form this means that at least the outer surfaces of the particles have to exhibit polar functional groups.

If the base material of a solid phase material is hydrophobic or not sufficiently hydrophilic, e.g. is based on a styrene or polyolefin (co)polymer, the surfaces that are to be in contact with an aqueous liquid may be hydrophilized. Typical protocols comprise that the solid phase material is coated with a compound or mixture of compounds exhibiting polar functional groups of the same type as discussed above,

Solid phase material in porous bed I (**104,204,304**) and porous bed II (**105,205,305**) is in principle selected amongst the same solid phase material as for AC_S in WO 04083108 (Gyros AB). Selection criteria or features based on the presence of bed-preserving agents may or may not be applied. An additional criterion for material in porous bed II (**104,204,304**) is that size exclusion materials in certain variants may be beneficial.

Porous bed II (**105,205,305**) may be considered as a filter for a liquid containing both a disturbing substance and solute S. In the case the disturbing substance is a particulate material it will be mechanically collected at or in the upper end of porous bed II (**105,205,305**). If the disturbing substance is in dissolved form there are two main choices for preventing the reactant to reach porous bed I (**104,204,304**) before or simultaneously with solute S: a) provide the solid phase material with an immobilized reactant R_{DS} that is capable of neutralizing the disturbing substance as indicted elsewhere in this specification, and/or b) provide the solid phase material in the form of a size exclusion material that delay the disturbing substance but not solute S.

It follows that properly selected size exclusion solid phase material may have a number of advantages since they, with the appropriate immobilized R_{DS}, will be active in neutralizing disturbing substances in the form of a) particulate material, b) compounds that are reactive with R_{DS}, and c) dissolved compounds that have smaller sizes than solute S. Preferred size exclusion material or media are liquid chromatography size exclusion material including gel filtration material. In this context "a smaller size" typically refers to a lower molecular weight and/or a smaller hydrodynamic size. Dissolved disturbing substances of low molecular weight may be found amongst substances that have molecular weights ≤ 100,000 daltons, such as ≤ 50,000 daltons or ≤ 10,000 daltons or ≤ 7,000 daltons. The terms "smaller size" and "molecular weight" given above refer to measurements done with chromatographic size exclusion material.

It is believed that proper size exlusion media are selected amongst solid phase materials that have Kav-values < 0.5, such as < 0.4 or < 0.1, for a reactant (solute S) that is to pass through porous bed II. Suitable size exclusion media should also have a Kav-value within the range of 0.1-0.95, typically within 0.40-0.95, if the goal is to delay small or low molecular weight disturbing substances or compounds that are not allowed to reach porous bed I before or simultaneous with solute S. For a definition of Kav see L. Hagel in "Protein Purification, Principles, High Resolution, and Applications", J-C Janson and L Rydén (Eds), VCH Publishers Inc. New York, 1989, p. 99.

The selection of immobilized reactant R_{DS} is done according to well known principles and depends on kind of disturbing substance. R_{DS} may thus be a) chemical reactant capturing the disturbing substance by covalent bonds to porous bed II **(105,205,305)** or otherwise making the substance harmless to the process, b) a component of a catalytic system that converts the substance to a harmless product, c) an affinity counterpart (AC_{DS}) to the disturbing substance. Heterophilic antibodies of analyte-containing samples may for instance be captured to porous bed II **(105,205,305)** according to (c) in the case AC_{DS} is an immunoglobulin preparation of irrelevant antibody activity (cold Ig) but exposing antigenic determinants reactive with antibody-active parts of the heterophilic antibodies. The solid phase material of porous bed II **(105,205,305)** may be a HIC-media, i.e. a hydrophilic separation medium comprising hydrophobic groups (=AC_{DS}) that potentially will interact and neutralize disturbing substances exposing hydrophobic groups such as lipoid-like substances and/or reagent fractions that are more hydrophobic than other reagent fractions.

Immobilization of R_{DS} may take place by techniques well-know in the field as discussed for the immobilized reagent R, such as an affinity counterpart to the solute (= AC_S) in porous bed II (**105,205,305**). The use of generic immobilizing affinity pairs (binder B_{DS} and ligand L_{DS}), such as streptavidin and biotin, respectively, can be envisaged to be particularly useful.

Further information about selection of immobilized R_{DS}, such as AC_{DS}, and immobilization techniques are found in WO 04083108 (Gyros AB) and in the discussion below about AC_S.

It can be envisaged that an immobilized amphiphilic macromolecular substance that typically is capable of forming micelles in water potentially may be particularly useful as an immobilized R_{DS} for removing disturbing effects from derivatized reagent molecules. Suitable such macromolecular substances are selected macromolecular substances exhibiting peptide structure, such as milk proteins e.g. caseins with preference for beta-casein. Suitable such substances may also be found amongst synthetic amphiphilic macromolecular substances such as tri block polymers comprising a central block of a hydrophobic polymer chain, e.g. a polypropylene oxide chain and end blocks of hydrophilic polymer chains, e.g. a polyethylene oxide. See further SE application 05001318 and corresponding US provisional application "Protecting agent" filed the 20th of January 2005. It is believed that the most significant improved effects for this kind of R_{DS}/AC_{DS} may occur with respect to downward extensions of dynamic ranges and limits of detection, and increased sensitivity in the lower part of a dynamic range. It is believed that this variant may be particular valuable for derivatized reagents, e.g. reagents in which hydrophobic groups, such as hydrophobic labels, have been introduced.

The solid phase material of porous bed I contains an immobilized reactant R that is capable of participating in organic, inorganic, biochemical interactions etc. Depending on the circumstances and the kind of reactant and solute S the interaction may be part of a a) separation, b) a catalytic reaction, c) an affinity reaction, d) a solid phase synthesis, and e) etc. Immobilized reactant R may in any of a)-e) be an affinity counterpart AC_S to solute S, i.e. capable of forming an affinity complex AC_S--S with solute S. Affinity bonds typically are based on: (a) electrostatic interactions, (b) hydrophobic interactions, (c) electron-donor acceptor interactions, and/or (d) bioaffinity binding.

Each of the affinity counterpart AC_S and solute S is thus a bioaffinity reactant/member of a bioaffinity pair. Typical bioaffinity pairs are a) antigen/hapten and an antibody, b) complementary nucleic acids, c) immunoglobulin-binding protein and immunoglobulin (for instance IgG or an Fc-part thereof and protein A or G), d) lectin and the corresponding carbohydrate, e) biotin and (strept)avidin/neutravidin, e) components of an enzymatic system (enzyme-substrate, enzyme-cofactor, enzyme-inhibitor etc), f) an IMAC group and an amino acid sequence containing histidyl and/or cysteinyl and/or phosphorylated residues (i.e. an IMAC motif), etc. Antibody includes antigen binding fragments and mimetics of antibodies. The term "bioaffinity pair" includes also affinity pairs in which one or both of the members are synthetic, for instance mimicking one or both of the members of a native bioaffinity pair. The term IMAC stands for an immobilized metal chelate.

The term "affinity reactant" also includes that solute S or AC_S may be a reactant that is capable of reversible covalent binding, for instance by disulfide formation. This kind of reactants typically exhibits a HS- or a -S-SO_n- group (n = 0, 1 or 2, free valences bind to carbon). See US 5,887,997 (Batista), US 4,175,073 (Axén et al), and 4,563,304 (Axén et al).

The immobilized reactant R may be a catalytic system as such or a component of a catalytic system. Components of catalytic systems are catalysts, cocatalysts, cofactors, substrates or cosubstrates, inhibitors, promotors etc. For enzymatic systems the corresponding components are enzymes, cocatalysts, cofactors, coenzymes, substrates, cosubstrates etc. The term "catalytic system" also includes linked catalytic systems, for instance a series of systems in which the product of the first system is the substrate of the second catalytic system etc and whole biological cells or part of such cells.

An immobilized affinity reactant (R, such as AC_S) should be selected to have the appropriate selectivity and specificity for interacting with solute S in relation to an intended application. General methods and criteria for the proper selection of reactant R are well known in the field.

The linkage to the solid phase material may be via covalent bonds, affinity bonds (for instance biospecific affinity bonds), physical adsorption etc. The techniques for immobilization are commonly known in the field.

Immobilization via affinity bonds may utilize a generic immobilizing affinity pair in which one of the members (immobilized ligand or L) is firmly attached to the solid phase material, for instance covalently. The other member (immobilizing binder, B) of the pair is used as a conjugate (immobilizing conjugate) comprising binder B and reactant (R e.g. AC_s). Examples of immobilizing affinity pairs are a) streptavidin/avidin/neutravidin and a biotinylated reactant (or vice versa), b) antibody and haptenylated reactant (or vice versa), c) an IMAC group and an amino acid sequence containing histidyl and/or cysteinyl and/or phosphorylated residues (i.e. an IMAC motif) linked to a reactant/solute S, etc. An immobilizing binding pair to be used in the invention is generic in the sense that the utilized affinity binding is irrelevant to subsequent affinity reactions.

The term "conjugate" primarily refers to covalent conjugates, such as chemical conjugates and recombinantly produced conjugates (where both the moieties have peptide structure). The term also includes so-called native conjugates, i.e. affinity reactants exhibiting two binding sites that are spaced apart from each other, with affinity directed towards two different molecular entities, for instance a native antibody that comprises species and class-specific determinants on one side of the molecule and antigen/hapten-binding sites on another side.

Preferred immobilizing affinity pairs (L and B) typically have affinity constants ($K_{L\text{-}B}$ = [L][B]/[L-B]) that are at most equal to or ≤ 10 times or 10^2 times or ≤ 10^3 times larger than the corresponding affinity constant for streptavidin and biotin. This typically will mean affinity constants that roughly are ≤ 10^{-13} mole/l, ≤ 10^{-12} mole/l, ≤ 10^{-11} mole/l and ≤ 10^{-10} mole/l, respectively. The preference is to select L and B amongst biotin-binding compounds and streptavidin-binding compounds, respectively, or vice versa, including as biotin-binding compounds anti-biotins such as avidin, streptavidin, neutravidin and other recombinatly chemically varied forms of avidin, streptavidin and neutravidin, anti-biotin antibodies.

The affinity constants discussed above refer to values obtained by a biosensor (surface plasmon resonance) from Biacore (Uppsala, Sweden), i.e. with the affinity reactant (AC_S and L) immobilized to a dextran-coated gold surface.

At least one member of an affinity pair, in particular a bioaffinity pair, to be used in the present invention may exhibit a structure selected amongst: a) amino acid structure including peptide structure such as poly and oligo peptide structure, b) carbohydrate structure, c)

nucleotide structure including nucleic acid structure, d) lipid structure such as steroid structure, triglyceride structure etc. The term affinity pair in this context refers to the immobilizing affinity pair (L and B), the affinity reactant and the solute (AC_S and S) and other affinity pairs that may be used for instance for immobilizing R_{DS}.

Flow paths and reaction microcavities/porous beds

A typical microchannel structure according to the invention comprises one, two, three or more flow paths (**101;201a,b;301a,a',b**) which each have a section (**108,208**) that is common to all of the flow paths. In this common section (**108,208**) there is a reaction microcavity I (**102,203,303**) containing a porous bed I (**104,204,304**) that also is common for all of the flow paths (**101;201a,b;301a,a',b**). Each flow path is used for transporting liquid through porous bed I (**104,204,304**). See figures 1-3. Upstream of the common section there is an upstream reaction microcavity (**102,202,202**) containing a porous bed II (**105,205,305**) in at least one of the flow paths (**101;201a;301a,a'**).

Porous bed I (**104,204,304**) exhibits a reactant R which is capable of interacting with solute S that is present in a liquid passing through the bed (**104,204,304**). Porous bed II (**105**) may or may not exhibit a reactant (R_{DS}) that is capable of interacting with a disturbing substance DS that is present in a liquid passing through the bed, for instance in a liquid containing solute S. Interaction between porous bed II (**105,205,305**) and the disturbing substance may also occur by size exclusion if the disturbing substance has a smaller size than solute S and the solid phase material of porous bed II (**105,205,305**) is appropriately selected.

Porous bed I (**104,204,304**) and/or porous bed II (**105,205,305**) may alternatively exhibit a generic ligand of a generic immobilizing affinity pair. In this variant the generic ligand may have the same binding specificity in at least two porous beds of a microchannel structure, e.g. porous beds I and II (**104,204,304** and **105,205,305**) in the same flow path may have essentially identical generic ligands (same ligands). Thus, both porous bed I (**104,204,304**) and porous bed II (**105,205,305**) in the same flow path (**101;201a;301a,a'**) may have biotin as a generic ligand (or an anti-biotin). Alternatively a generic ligand may have different binding specificity in porous beds I and II (**104,204,304** and **105,205,305**) in the same flow path. Thus, porous bed I (**104,204,304**) may have biotin and porous bed II (**105,205,305**) an anti-biotin or vice versa as generic ligands L in the same flow path (**101;201a;301a,a'**).

A reaction microcavity comprising porous bed I or II (**104,204,304** and **105,205,305**, respectively) is defined as the volume occupied by the bed. These reaction microcavities may like microcavites in general be a straight or bent microconduit that may or may not be continuously widening and/or narrowing. A microcavity that is located at the same position and/or function in one microchannel structure typically has essentially the same shape and/or size as the corresponding microcavity in another microchannel structure. As for reaction microcavities in general, a microcavity containing porous bed I or II (**104,204,304** and **105,205,305**) has at least one cross-sectional dimension that is ≤ 1,000 μm, such as ≤ 500 μm or ≤ 200 μm (depth and/or width). The smallest cross-sectional dimension is typically ≥ 5 μm such as ≥ 25 μm or ≥ 50 μm. The total volume of a reaction microcavity is typically in the nl-range, such as ≤ 5,000 nl, such as 1,000 nl or ≤ 500 nl ≤ 100 nl or ≤ 50 nl or ≤ 25 nl.

Figure 1a illustrates variants in which there is at least one flow path (**101**) with one single reaction microcavity (**102+103**) for both porous bed I (**104**) and porous bed II (**105**). The downstream end of the upstream bed (**105**) is abutted to the upstream end of the downstream bed (**104**), possibly including a porous membrane (**106**) between the beds (**104,105**).

Figure 1b illustrates variants in which there is at least one flow path (**101**) with two separate microcavities I and II (**102** and **103**, respectively) – one for porous bed I (**104**) and one for porous bed II (**105**). As discussed in more detail for figure 2 there may be a liquid router function (**107**) between the two reaction microcavities (**102** and **103**). This router (**107**) is able to guide liquid into either a micrconduit (**109**) leading to porous bed I (**104**) or a microconduit (**110**) that permits liquid to exit the flow path (**101**)

Figure 2 illustrates variants in which there are at least two kinds of flow paths (**201a** and **201b**). One of them comprises flow paths (**201a**) in which both porous beds I and II (**204** and **205**) are present as described above. The other ones comprise flow paths (**201b**) that are devoid of porous bed II. There may be a liquid router function (**207**) between the reaction microcavities/beds (**202/204;203/205**). This function will permit switching liquid to either of the two microconduits A and B (**209** and **210**, respectively) and make it possible to prevent a liquid that is intended to interact only with porous bed II (**205**) from passing through porous bed I (**204**). The liquid router function (**207**) has a valve function that is mechanical or is based on surface tension. If centrifugal force is used, the routing function may be as described in PCT/SE2004/001424 (Gyros AB). This liquid router function may be advantageous in the

case porous bed II (**205**) initially contains a generic ligand to which one immobilizes reactant R_{DS} in the form of a conjugate containing a generic affinity counterpart (binder) to the generic ligand. Immobilization typically takes place by transporting the liquid containing the conjugate through porous bed II (**205**) and out through exit microconduit B (**210**). In the case porous bed I (**204**) exhibits a generic ligand, the immobilized reactant R is introduced by passing reactant R in the form of a conjugate with a generic counterpart to this ligand (generic binder) via the flow path (**201b**) through porous bed I (**204**) i.e. via a flow path that is devoid of porous bed II (**205**). When performing the actual method the liquid that contains a reactant together with the disturbing substance is passed through bed II (**205**) and via microconduit A (**209**) through porous bed I (**204**). Other liquids may preferably be introduced via flow path (**201b**) in which there is no porous bed II.

Figure 3 illustrates another variant in which there are at least two kinds of flow paths (**301a,301a'** and **301b**). One of them comprises flow paths (**301a,301a'**) in which both porous beds I and II (**304 and 305**) are present. The other one (**301b**) comprises flow path only containing porous bed I (**304**) that is common for all the flow paths (**301a,301a'** and **301b**). In each flow path (**301a,301a'**) there may be a liquid router (**307,307'**) between porous bed II (**305,305'**) and porous bed I (**304,304'**) with a microconduit (309) leading to porous bed I, and an exit microconduit B (**310**). Porous bed II (**305**) in one or more of the flow paths (**301a**) containing porous bed II may be different from porous bed II (**305**) in one or more of remaining ones of the flow paths (**301a'**) containing porous bed II, or essentially identical. The difference may relate to kind of matrix, immobilized reactant (R_{DS}) interacting with a disturbing substance, generic ligand, etc. This variant is particularly useful if one liquid contains a first disturbing substance and the other liquid a second different disturbing substance that can not be neutralized by the use of the same solid phase material, for instance the same immobilized reactant R_{DS}.

This general outline of microchannel structures that can be used in the present invention can be further illustrated by figure 4c in WO 02074438 (Gyros AB), figure 1 of WO 04083108 (Gyros AB), and figure 2 in PCT/SE2004/001424 (Gyros AB). The structure used in experiment 1 and 2 in this specification was according to figure 1 of WO 04083108 (Gyros AB).

The flow paths described above only represent a part of a microchannel structure. In addition there may be one, two, three or more functional units selected among: a) inlet arrangement comprising for instance an inlet port/inlet opening, possibly together with a volume-metering unit, b) further microconduits for liquid transport, c) one or more other reaction microcavities, e.g. for performing homogeneous reactions; d) mixing microcavity; e) unit for separating particulate matters from liquids (may be present in the inlet arrangement), f) unit for separating dissolved or suspended components in the sample from each other, for instance by capillary electrophoresis, chromatography and the like; g) detection microcavity; h) waste conduit/microcavity; i) valve; j) vent to ambient atmosphere; etc. A functional part may have more than one functionality, e.g. a reaction microcavity containing a porous bed I (**104,204,304**) and a detection microcavity containing porous bed I (**104,204,304**) may coincide. Various kinds of functional units in microfluidic devices have been described by Gyros AB/Amersham Pharmacia Biotech AB: WO 9955827, WO 9958245, WO 02074438, WO 0275312, WO 03024598, WO 03018198 and by Tecan/Gamera Biosciences: WO 0187487, WO 0187486, WO 0079285, WO 0078455, WO 0069560, WO 9807019, WO 9853311.

Many of these functional units may be present in a microchannel structure used in the invention, for instance upstream porous bed II (**105,205,305**), between porous beds II and I (**105,205,305 and 104,204,304**), and/or downstream porous bed I (**104,204,304**) in order to further process liquid aliquots transported in a flow path/microchannel structure. A microchannel structure may also contain additional porous beds upstream or downstream the porous beds utilised according to main characteristics of the invention. Additional pairs of porous beds I and II in a microchannel structure may define sets of flow paths that are separate from the flow paths (**101;201a,b;301a,a',b**).

As discussed above a reaction microcavity intended for a porous bed may be connected to one or more inlet arrangements (upstream direction), each of which comprises an inlet port and at least one volume-metering unit. In one advantageous variant, there is one separate inlet arrangement per microchannel structure and reaction microcavity intended to contain the solid phase material. In another advantageous variant, the inlet arrangement is common to all or a subset of microchannel structures (and thus also to a subset of reaction microcavities). This kind of common inlet arrangement typically comprises a common inlet port and a distribution manifold with one volume-metering unit for each microchannel structure/reaction microcavity

of the subset. In both variants, each of the volume-metering units in turn is communicating with downstream parts of a microchannel structure and/or a downstream reaction microcavity. Microchannel structures linked together by a common inlet arrangement and/or common distribution manifold define a group of microchannel structures of a device.

Typical inlet arrangements have been presented in WO 0274438 (Gyros AB), WO 0275312 (Gyros AB), WO 0275775 (Gyros AB) and WO 0275776 (Gyros AB).

The microfluidic device may also comprise other common microchannels/microconduits connecting different microchannel structures. Common channels including their various parts such as inlet ports, outlet ports, vents, *etc.*, are considered part of each of the microchannel structures they are communicating with.

Other features of a microfluidic device

A microfluidic device is a device that comprises a microchannel structure in which one or more liquid aliquots in the µl-range, typically in the nanolitre (nl) range, containing various kinds of reactants, such as analytes and reagents, products, samples, buffers and/or the like are processed. A liquid aliquot in the µl-range have a volume ≤ 1,000 µl, such as ≤ 100 µl or ≤ 10 µl and includes the nl-range that has an upper end of 5,000 nl but in most cases relates to volumes ≤ 1,000 nl, such as ≤ 500 nl or ≤ 100 nl. The nl-range includes the picolitre (pl) range. A microchannel structure comprises one or more cavities and/or conduits that have a cross-sectional dimension that is ≤ 10^3 µm, preferably ≤ 5 x 10^2 µm, such as ≤ 10^2 µm.

A microfludic device preferably contains a plurality of microchannel structures/device intended to contain the solid phase according to the invention. Plurality in this context means two, three or more microchannel structures and typically is ≥ 10, e.g. ≥ 25 or ≥ 90 or ≥ 180 or ≥ 270 or ≥ 360. As discussed above the microcannel structures of a device may be divided into groups, each of which may be defined by the size and/or shape of the reaction microcavity, by a common microchannel, such as a common inlet arrangement or manifold etc. Each such group typically comprises from 3-15 or 3-25 or 3-50 microchannel structures.

Different principles may be utilized for transporting aliquots of liquid within the microfluidic device/microchannel structures between two or more of the functional parts described above. Inertia force may be used, for instance by spinning the disc as discussed below. Other

favourable forces are capillary forces, electrokinetic forces, non-electrokinetic forces such as capillary forces, hydrostatic pressure *etc.*

The microfluidic device typically is in the form of a disc. The preferred formats have an axis of symmetry (C_n) that is perpendicular to the disc plane, where n is an integer ≥ 2, 3, 4, 5 or more, preferably ∞ (C_∞). In other words the disc may be rectangular, such as squaric, and other polygonal forms but is preferably circular. Once a proper disc format has been established, centrifugal force may be used for driving liquid flow by spinning the device around a spin axis. The spin axis may be perpendicular or parallel to the disc plane. In preferred variants the spin axis coincides with an axis of symmetry.

For preferred centrifugal-based variants, each microchannel structure comprises one upstream section that is at a shorter radial distance than a downstream section relative to a spin axis. A reaction microcavity containing porous bed I and/or II is then at a radial position that is intermediary to the radial positions of the two sections.

The preferred devices are typically disc-shaped with sizes and forms similar to the conventional CD-format, e.g. sizes that are in the interval from 10% up to 300 % of the conventional CD-radii.

Microchannels/microcavities of a microfluidic devices may be manufactured from an essentially planar substrate surface that exhibits the channels/cavities in uncovered form that by covering the surface with another essentially planar substrate (lid). See WO 9116966 (Pharmacia Biotech AB) and WO 0154810 (Gyros AB). Both substrates are preferably fabricated from plastic material, e.g. plastic polymeric material.

The fouling activity and hydrophilicity of inner surfaces should be adapted to the process to be carried out in the device. See for instance WO 0147637 (Gyros AB).

The terms "wettable" (hydrophilic) and "non-wettable" (hydrophobic) contemplate that a surface has a water contact angle ≤ 90° or ≥ 90°, respectively. In order to facilitate efficient transport of a liquid between different functional parts, inner surfaces of the individual parts of a microchannel structure should primarily be wettable, preferably with a contact angle ≤ 60° such as ≤ 50° or ≤ 40° or ≤ 30° or ≤ 20°. These wettability values apply for at least one,

two, three or four of the inner walls of a microconduit. In the case one or more of the inner walls have a higher water contact angle this can be compensated for by a lower water contact angle for the remaining inner wallS. The wettability, in particular in inlet arrangements should be adapted such that an aqueous liquid will be able to fill up an intended microcavity by capillarity (self suction) once the liquid has started to enter the cavity. A wettable surface of an inner wall of a microchannel structure may comprise one or more local hydrophobic surface breaks in a hydrophilic inner side-wall, for instance for introducing a passive valve, an anti-wicking means, a vent solely function as a vent to ambient atmosphere etc. See for instance WO 9958245 (Gyros AB) and WO 0274438 (Gyros AB).

Contact angles refer to values at the temperature of use, typically +25°C, are static and can be measured by the method illustrated in WO 0056808 (Gyros AB) and WO 0147637 (Gyros AB).

Methods utilizing the microfluidic device

The second aspect of the invention is a method for carrying out a microfluidic process in a flow path of a microchannel structure as defined for the first aspect of the invention. This microfluidic process typically requires that there are additional functionalities/parts in the microchannel structure such as additional flow paths containing or not containing porous beds of the type required by the method of the invention. For each of at least one, two or more of the microchannel structures of a device, the method comprises the steps of:

(i) providing a first liquid aliquot containing a solute S in a position that is upstream a porous bed II (**105,205,305**) of a flow path (**101,201a,a'..,301a,a'..**) which comprises both porous bed II (**105,205,305**) and porous bed I (**104,204,304**),

(ii) transporting the aliquot through porous bed II (**105,205,305**), and

(iii) transporting solute S subsequently through porous bed I (**104,204,304**).

Step (i) comprises that the aliquot and/or solute S is formed within the device/microchannel structure or is dispensed to the microchannnel structure. Formation of the aliquot and/or of solute S is typically in a position upstream porous bed II (**105,205,305**). Dispensing of the aliquot is to a position upstream porous bed II (**105,205,305**) and via an inlet port of each of microchannel structures utilized in the method. This inlet port is in liquid communication with the flow path at a position that is upstream porous bed II (**105,205,305**). The functionality in

which formation is taking place or the inlet port to which the aliquot is dispensed may be common to several microchannel structures.

The sequence (i)-(iii) typically means that an immobilized reactant in porous bed I (**104,204,304**) is neutralized, for instance transformed to another group or compound that then may become available

a) as an immobilized product that can be used as reactant in a subsequent step of the process, for instance a subsequent run of the sequence (i)-(iii), or

b) as a product released from porous bed I (**104,204,304**) and used in other steps of the process.

Porous beds I and II (**104,204,304;105,205,305**) that comprise immobilized reactants (R and R_{DS}) that are non-substrate components of catalytic systems may be reusable in repetitive runs of the sequence (i)-(iii). An analytically detectable product/reactant formed as described may be used as an indicator for characteristics of the process, for instance reflecting characteristics of a solute S that is an analyte. Analytes can thus be determined from values obtained by measuring this kind of products/reactants if the process is analytical if there is an analyte to be characterized. The measurement may take place in reaction microcavity I (**102,202,302**)/ porous bed I (**104,204,304**) and/or downstream this microcavity/porous bed.

The sequence (i)-(iii) may be repeated once, twice or more as required by the process to be carried out. This means that the process and also the method may comprise a second run of steps (i)-(iii) in which a second liquid aliquot is used. This second aliquot typically contains another solute S, one or more disturbing substances that possibly are different from the disturbing substance of the first aliquot, other buffers or buffer concentrations and/or a different solvent composition, etc. The second aliquot is provided in the same flow path (**301a**) as the first aliquot or in another flow path (**301a'**) containing both porous beds I and II. The ability of the porous bed II in this other flow path (**301a'**) to interact with disturbing substances is typically different from the corresponding ability of the porous bed II (**105,205,305**) in the first utilized flow path (**301a**). Still further aliquots (3^{rd}, 4^{th} etc) containing solute S and disturbing substances may subsequently be provided in subsequent repetitive runs of the sequence (i)-(iii). For each of the additional aliquots/runs (2^{nd}, 3^{rd}, 4^{th}....) porous bed II (**105,205,305**) and flow path is selected to be able to hinder disturbing substances to reach porous bed I (**104,204,304**) simultaneously with or before solute S of the aliquot transported.

Before the first run, between runs and/or subsequent to the last run of the sequence (i)-(iii), each of one or more additional liquid aliquots may be provided

a) upstream porous bed II (**105,205,305**) in a flow path (**101,201a,301a,a'**) containing porous bed II (**105,205,305**), or

b) upstream porous bed I (**104,204,304**) in a flow path (**201b,301b**) not containing porous bed II (**105,205,305**).

These additional aliquots are typically devoid of disturbing substances including that they may contain disturbing substances that for various reasons have been considered acceptable for the process. They are typically washing or conditioning liquids lacking a solute S and possibly containing buffering substances, water, water-miscible solvents, and surface active agents such as detergents and/or tensides. One or more of them may contain a solute S.

The method and the process according to the invention may also comprise processing of liquid aliquots that do not require that the aliquots are passed through porous beds I (**104,204,304**) and II (**105,205,305**). Typical such steps are processing downstream porous bed I (**104,204,304**) and upstream porous bed II (**105,205,305**).

The transport during steps (ii) and (iii) in the first and/or in a repetitive run may be selected to provide static or flow conditions for the interaction between

a) the immobilized reactant R_{DS}, such as affinity counterpart AC_{DS}, and the disturbing substance, and/or

b) immobilized reactant R, e.g. affinity counterpart AC_S, and solute S.

Typical flow rates through a porous bed for flow conditions provide a residence time ≥ 0.010 seconds such as ≥ 0.050 sec or ≥ 0.1 sec with an upper limit that typically is below 2 hours such as below 1 hour. Illustrative flow rates are within 0.01-1000 nl/sec, such as 0.01-100 nl/sec and more typically 0.1 - 10 nl/sec. These flow rate intervals may be useful for bed volumes in the range of 1-200 nl, such as 1-50 nl or 1-25 nl. Residence time refers to the time it takes for a liquid aliquot to be in contact with the solid phase in the reaction microcavity. The flow rate may be adapted such that the interactions referred to are taking place under diffusion-limiting or non-diffusion limiting conditions. Further details and advantages with flow conditions are given in WO 0275312 (Gyros AB).

The sequence (i) – (iii) may be part of (a) separation method, (b) a method of catalysis, (c) a solid phase synthesis etc.

Separation comprises among others:

a) capturing, i.e. porous bed I exhibits an affinity structure (affinity ligand, affinity reactant) with binding ability for a solute S such that when a liquid containing solute S passes through the bed then a liquid without solute S or with a reduced amount of solute S will appear in the eluate, and/or

b) immobilization of the solute on the solid phase material for later use of the solid phase material so modified in capturing, catalytic reactions, solid phase synthesis etc.

For alternative (a) (capturing), the solute becomes bound to porous bed I (**104,204,304**) and forms an immobilized affinity complex comprising the affinity structure and the solute.

For immobilization (alternative b), porous bed I (**104,204,304**) may exhibit a generic affinity structure L' (generic ligand) (= AC_S) that is an affinity counterpart to a conjugate B'-AC'_S (= S') which has two kinds of binding sites – the first one B' is generic and directed towards generic affinity structure L' on porous bed I (**104,204,304**) and the second one AC'_S towards a solute S. L', B', S' and AC'_S then correspond to L, B, S and AC_S above and what has been said in the context of the first aspect of the invention for L, B, S and AC_S including affinity constants also applies here.

A separation may be part of a purification or an enrichment protocol for solute S. Solute S may be a contaminant or the entity to be purified, enriched etc. The separation may also be part of a synthetic protocol, preparative protocol, a cell based assay, various kinds of affinity assays including nucleic acid assays, immunoassays, enzyme assays and other ligand-receptor assays based on the affinity between a receptor and a ligand.

An affinity assay that utilizes a capturing step for binding solute S to a reactant R immobilized on a solid phase material (porous bed I (**104,204,304**)) typically aims at the characterization of an uncharacterized feature or variable of a reactant (= analyte) utilized in the assay. Typical features/variables are of two kinds: a) amounts including presence and/or absence, concentration, relative amounts, activity such as binding activity and enzyme activity, etc, and b) properties of affinity reactants including affinity as such, e.g. affinity

constants, specificities etc. Reaction variables such as pH, temperature, ionic strength etc and their influence on the outcome of the experiments/reactions at issue may also be characterized/determined (yield, precision, recovery etc). See WO 02705312 (Gyros AB).

Two major groups of affinity assays to which the present invention can be applied are a) competitive affinity assays including also inhibition and displacement assays, or b) non-competitive affinity assays including sandwich assays. The conditions in an affinity assay are selected so that the amount of the affinity complex is correlated with the absence, the presence and/or the amount (including concentration) of an analyte in a sample. An affinity assay may be heterogeneous in the sense that it in at least one step utilizes an affinity reaction between an immobilized reactant and an affinity counterpart in order to form an affinity complex. An affinity assay may utilize an analytically detectable reactant to measure an affinity complex, the amount of which correlates with the absence, the presence and/or the amount of an analyte in a sample. An analytically detectable reactant used in an affinity assay may be inherently detectable or be a man-made conjugate between an affinity reactant and a label compound. There are two major kinds of labels:

a) signal-emitting labels such as enzymatic labels (enzyme, coenzyme, substrate, cosubstrate, cofactor et), radioactive isoptopes, chromophors and/or chromogens, fluorophors and/or fluorogens, bioluminophors and/or bioluminogens, chemiluminophlors and/or chemiluminogens, metal atoms and ions, etc, and
b) affinity labels that typically require secondary detectable reactants comprising one part that is capable of binding to an affinity label and a another part that is analytically detectable.

A catalytic reaction as applied to the present invention comprises that the solid phase material (porous bed I (**104,204,304**)) exhibits one or more immobilized components (affinity structure, affinity ligand, affinity reactant) of the catalytic system utilized, while one of the other components of the same system is solute S. The catalytic reaction comprises formation of an affinity complex between the immobilized member (affinity structure, affinity ligand, affinity reactant) and solute S plus possibly also other members of the catalytic system.

The term "catalytic system" includes single catalytic system and more complex variants comprising a series of linked single enzyme systems, single catalytic systems linked to other reaction systems, whole cells, cell parts exhibiting enzymatic activity etc. The bed may

function as a catalytic reactor, such as an enzyme reactor. Enzyme receptor-ligand assays (enzyme affinity assays), such as enzyme immunoassays are examples of processes that utilizes linked catalytic systems, e.g. linked to other receptor-ligand reactions.

The step during which interaction between the immobilized reactant R and solute S occurs may be part of a catalytic assay, such as an enzyme assay, for characterizing one or more members of the catalytic system or some other reaction variable (e.g. reaction condition). The assay may be for determining the activity of a particular catalyst, substrate, co-substrate, cofactor, co-catalyst etc in a liquid sample. The term catalytic assay also contemplates so called enzyme immuno assays. The molecular entity/entities corresponding to the activity to be determined is/are called analyte/analytes. See WO 03093802 (Gyros AB).

Solid phase synthesis includes for instance polymer synthesis, such as oligopeptide and oligonucleotide synthesis and synthesis of other small molecules on a solid phase material. The immobilized reactant used in polymer synthesis, for instance, may exhibit the structure of the corresponding monomer, such as nucleotide, carbohydrate, amino acid structure, and mimetics of these structures. Synthesis of libraries of immobilized members of combinatorial libraries is also included. Such members have relatively low molecular weights (e.g. < 10,000 dalton including a possible spacer to a polymeric backbone).

Microfluidic devices comprising generic ligands

The third aspect of the invention comprises a microfluidic device in which there are one, two or more microchannel structures. At least one of these microchannel structures comprises one, two or more flow paths (**101,201a,a',301a,a',b**) and each of at least one, two or more of these flow paths (**101,201a,a',301a,a'**) comprises two porous beds (**104,204,304;105,205,305**) in series as described for the first aspect. As for the first aspect there may be additional functional units upstream of the upstream porous bed (**105,205,305**), between the porous beds (**104,204,304;105,205,305**), and downstream of the downstream porous bed (**104,204,304**).

The characteristic feature is that in each flow path that comprises the upstream porous bed and the downstream porous bed, at least one, preferably both, of the beds comprises/comprise a generic ligand L that may be the same or different in the two beds. This ligand is in each bed immobilized to the solid phase material of the bed. The two ligands in a flow path are represented with L_I for the downstream bed (**104,204,304**) and L_{II} for the upstream bed

(**105,205,305**). In the case there are two or more flow paths comprising the two porous beds the combination of generic ligands may be different between at least two of the flow paths, but preferably the combination is the same in all flow paths containing porous bed I and porous bed II.

Typical combinations of ligands in a flow path comprising bed I and bed II are: 1) $L_I = L_{II}$, 2) L_{II} = anti-L_I, and 3) L_I = anti-L_{II}. For biotin and biotin-binding compounds (= anti-biotins) as generic immobilizing affinity pair this means: 1) $biotin_I$;$biotin_{II}$ or $anti_I$-biotin;anti-$biotin_{II}$, 2) $biotin_I$;anti-$biotin_{II}$, and 3) anti-$biotin_I$;$biotin_{II}$.

The binding capacity of porous bed I or II (**104,204,304;105,205,305**) for a generic ligand L can be measured as the amount of ligand in mole per unit volume, disregard blocking and destruction of binding sites caused by the immobilization. With this measure suitable binding capacities will typically be found within the interval of 0.001 - 3000 pmole, such as 0.01 - 300 pmole, per nl solid phase in bed form saturated with liquid. For instance, if 0.1 pmole streptavidin per nl has been immobilized this corresponds 0.4 pmole/nl biotin-binding sites. The conversion factor four is because streptavidin has four binding sites for biotin per streptavidin molecule. Binding capacity can also be measured as actual binding capacity for binder B, i.e. mole active binding sites per unit volume of the solid phase containing the immobilized affinity ligand in bed form saturated with liquid. This kind of binding capacity will depend on the immobilization technique, the pore sizes of the solid phase, the size of the entity to be immobilized, the material and design of the solid phase etc. Ideally the same ranges apply for the actual binding capacity as for the total amount of binding sites (as defined above). The actual binding capacity primarily refers to binding/capturing of the binder B in its basic form, e.g. unconjugated and/or underivatized. See also WO 04083109 (Gyros AB).

Other characteristic features of the third aspect of the invention with respect to microchannel structures/microfluidic devices including various flow paths, microcavities, functional units etc, solid phase material etc may be as discussed for the third aspect of the invention.

EXPERIMENTAL PART

EXPERIMENT 1

COMBINATION OF A DUMMY POROUS BED WITH A POROUS BED EXPOSING ANTI-ANALYTE ANTIBODY COMPARED TO A SOLE POROUS BED EXPOSING ANTI-ANALYTE ANTIBODY.

This example describe a sandwich immunoassay for PDGF β-receptor in cell lysates from porcine aorta endothelial (PAE) cells stably expressing the membrane bound PDGF β-receptor and stimulated with +/- ligand PDGF-BB. The capture antibody was raised against the target protein and the detection antibody against a regulatory phosphorylated amino acid site. The assay including immobilization, labeling (Alexa 647) and microchannel structure/microfluidic device/instrument is the same as for the myoglobin assay inWO 04083108 (Gyros AB). The microchannel structure used is given in figure 1 of WO 04083108. The porous bed was placed in reaction microcavity (104).

Cell culture: PAE cells were transfected with PDGF β-receptor and one of the cell cultures were also G-418 selected [Claesson-Welsh, L. et al. *cDNA cloning and expression of a human platelet-derived growth factor receptor specific for B-chain containing PDGF molecules.* Mol. Cell. Biol. **8** (1988) 3476-3486]. The cells were grown in Ham's F-12 medium supplemented with 10% fetal calf serum, 100 μg/ml streptomycin and glutamine. A nearly confluent monolayer of cells were starved overnight in Ham's F-12 supplemented with 0.1 mg/ml bovine serum albumin (BSA) and +/- stimulated with 100 ng/ml PDGF-BB for 60 minutes on a shake plater. Unstimulated cells are used as a control since the cells only have unphosphorylated PDGF β-receptors. The receptors are activated and saturated with high concentration of ligand inhibiting internalisation followed by degradation during the stimulation. After the stimulation, cells were washed twice in ice-cold PBS buffer and scraped off with a "rubber police men" in 1 ml PBS. The cell suspension were saved and lysed in 200 μl ice-cold lysis buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 mM EDTA, 0.5% Triton X-100, 0.5% Deoxycholate, 0.5 mM Na_3VO_4 and 1% Trasylol (Bayer) for 15 min on ice. The lysates were centrifuged at 13000 rpm for 15 min at 4°C and the supernatant were saved in aliquots and stored at –20°C. The the cells were in a first grown in 75 cm^2 culture dishes and in a second run in 175 cm^2 culture flasks in order to make more concentrated lysates. Total protein was quantitated according to BCA Protein Assay Kit Microplate procedure from PIERCE Biotechnology (Boule Nordic AB, Huddinge, S.).

PDGF β-receptor antibodies: Rabbit polyclonal antibody 958 raised against a recombinant protein corresponding to amino acids 958-1106 of the carboxy terminus of human PDGF β-receptor, goat polyclonal antibody P-20 raised against a peptide of the carboxy terminal of human PDGF β-receptor, and mouse monoclonal PY99 were from Santa Cruz Biotechnologies (Santa Cruz, CA). The PDGF β-receptor antibodies P-20 and 958 are recommended for the detection of PDGF receptor type β of human and, to a lesser extent, of mouse and rat origin by western blotting, immunoprecipitation and immunohistochemistry. They should not be cross-reactive with PDGF receptor type α. The antibodies have been used extensively in immunoprecipitation and Western Blot experiments with the same cell culture [Pietras K., et al. Inhibition of PDGF Receptor Signaling in Tumor Stroma Enhances Antitumor Effect of Chemotherapy. Cancer Research 62 (2002) 5476-5480].

Sandwich-based immunoassay methods: In the assay, wash buffers and antibodies were distributed through the common distribution channel and cell lysate preparations through the individual inlets. Every batch run included standards in triplicates and blank samples. The assay included a small dilution series (+/- PDGF-BB) and several blanks with lysis buffer. All steps in the assay were automatically run in the Gyrolab Workstation (Gyros AB).

In the assay different combinations of antibodies were tested to find the combination giving the highest degree of specific binding, 958/PY99, 958/P-20, P-20/P-20, P-20/958 and P-20/PY99 (capturing/detecting).

Titration showed that the concentration of the detection antibody should be 400 nM.

The β-PAE cell lysate with +/- PDGF-BB stimulation was diluted in lysis buffer (2 x, 4 x, 8 x). Undiluted cell lysate was included as a standard point.

Immobilization of capture antibody: The porous beds were washed twice with PBS-T (0.015 M Na-PO_4 pH 7.4, 0.15 M NaCl, 0.01% NaN_3, 0.01% Tween-20) followed by a short spin to recondition the polystyrene beads coated with phenyldextran to which streptavidin has been immobilized. Biotinylated capture antibody diluted in the wash buffer was added at a concentration of 667 nM followed by a spin. Washing was repeated twice in the same manner as the reconditioning.

Basic assay protocol: The cell lysate was added to individual inlets, volume defined (200 nl) and transported into the porous beds where PDGF β-receptor (analyte) was captured. After two wash steps followed by a short spin to ensure that the beds were filled with liquid during the fluorescence detection. The background fluorescence detection included three detection steps with different sensitivity set on the LIF detector, 1%, 5% and 25%. Excess buffer was washed away by a short spin before addition of the detection antibody which was allowed to bind to the analyte during a spin step. Six wash steps were finally included, two with normal wash buffer and four with wash buffer containing isopropanol 20%, to remove excess detection antibody.

In the beginning, cell lysate runs were mainly performed according to the basic assay protocol with the capture anti body being distributed all over the porous bed. Modifications were tested because of difficulties to establish a specific interaction between the PDGFβ-receptor and the antibodies. First of all, the analyte and the detection antibody spin program were extended and the linear flow over the bed reduced to enable the PDGF β-receptor in the cell lysate to attach to the capture antibody. After addition of analyte, three extra wash steps were included with PBS buffer without Tween-20 and the second and the fourth wash step were followed by a pulsed spin program, allowing the solution to move through the beds by simple diffusion. The final wash steps after the detection antibody were the same as in the preceding paragraph with the exception that isopropanol was excluded in the wash buffer.

Due to uncontrolled non-specific interactions in the cell lysate assay a second porous bed (II) devoid of capture anti-analyte antibody was loaded on top of the porous bed (I) exposing anti-analyte antibody. Porous bed (II) consisted of the gel filtration media Superdex™ peptide (Amersham Biosciences, Uppsala, Sweden). It media was loaded in a 5 x dilution slurry onto porous bed I (polystyrene beads coated with phenyldextran to which streptavidin has been immobilized). Superdex™ peptide is used for high-resolution gel filtration of peptides and other small biomolecules of 100-7000 daltons in molecular weight.

RESULTS

From pre-experiments three antibody pairs were selected P-20/958, P-20/PY99 and 958/PY99.

Porous bed (I) solely. The cell lysate experiments resulted in high CV values due to the signal distribution (triplicates) in the bed. In some experiments a small difference in signal could be seen between undiluted cell lysate and blank signals but mostly significant signals were hard to distinguish from background signals. The enrichment in the beds was mostly irregular and a general bed pattern could not be seen, in some cases the signal had a tendency to progress down the porous bed.

Porous bed (II) upstream of porous bed (I). Two combinations were tested:

a) a bed of polystyrene beads (essentially non-porous) coated with phenyldextran on top of a bed of polystyrene particles coated with phenyldextran to which streptavidin has been immobilized, and

b) a bed of Superdex™ peptide on top of a bed of polystyrene beads coated with phenyldextran to which streptavidin has been immobilized.

The Superdex™ peptide combination showed improvement in responses for the three antibody pairs compared to the single bed variant. Differences between cell lysate and blank responses could also be discerned.

EXPERIMENT 2

Neutralization of effects of heterophilic antibodies in native samples.

Basic assay: The microfluidic sandwich fluorescence immuno assay for TNFα was as outlined for myoglobin in the experimental part of WO 04083108 (Gyros AB). The solid phase and the immobilization technique and the fluorescence labelling technique were also as outlined WO 04083108 (Gyros AB). The assay was modified by placing an IgG-bed or the corresponding bed devoid of immobilized IgG (in control experiments) immediately upstream of the bed exposing capture anti-TNFα mouse antibody.

Instrument and microfluidic device: Both were the same as used in WO 04083108 (Gyros AB).

Samples: Three human serum samples rich in heterophilic antibodies and one normal were used in A. All four samples were spiked with human TNFα (200 pg/ml) and used in B.

Solid phase - Immunglobulin: The same kind of streptavidin particles as used in WO 04083108 (Gyros AB) were in a batch mode coated with four combinations of bovine IgG och mouse IgG - bovine IgG:mouse IgG 10:1; bovine IgG:mouse IgG 1:1; bovine IgG; mouse IgG. The particles were then loaded on top of a bed exposing capture anti-TNFα mouse antibody. The loading technique for both beds was the same as outlined for the anti-myoglobin antibody bed in WO 04083108 (Gyros AB). The flow direction during the experiment is from the IgG-bed to the anti-TNFα mouse antibody bed.

Experiments: Effects of an IgG-bed in front of the anti-TNFα mouse antibody bed on A) assaying heterophilic antibodies in four human serum samples given above, B) Recovery of TNFα for the TNFα assay of TNFα spiked human serum samples given above, and C) standard curve.

Results of (A): See figure 4. The staples in each group represent individual samples. It is apparent that the IgG-bed based on a bovine:mouse IgG of 10:1 was most efficient in capturing heterophilic antibodies. No heterophilic antibodies were detected in the normal serum sample. Without the use of an IgG-bed in front of the anti-TNFα mouse antibody bed the latter bed captured large amounts of heterophilic antbodies that would disturb a TNFα assay based on the anti-TNFα mouse antibody bed.

Results of (B): See figures 5a. The staples in each group represent individual samples, again with no staples for the normal serum sample. The change in recovery of TNFα when placing an IgG-bed in front of the anti-TNFα mouse antibody bed was acceptable.

Results of (C): See figure 5b. The change of the standard curve was insignificant when placing different IgG-bed in front of the anti-TNFα mouse antibody bed compared to without an IgG-bed.

Certain innovative aspects of the invention are defined in more detail in the appending claims. Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined by the appended claims. Moreover, the scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, composition of matter, means, methods and steps described in the specification. As one of ordinary skill in the art will readily appreciate from the disclosure of the present invention, processes, machines, manufacture,

compositions of matter, means, methods, or steps, presently existing or later to be developed that perform substantially the same function or achieve substantially the same result as the corresponding embodiments described herein may be utilized according to the present invention. Accordingly, the appended claims are intended to include within their scope such processes, machines, manufacture, compositions of matter, means, methods, or steps.

CLAIMS

1. A microfluidic device that comprises a microchannel structure in which there are one, two or more flow paths (**101;201a,b;301a,a',b**) all of which comprises a porous bed I (**104,204,304**) that is common for all of the flow paths, which bed exposes an immobilized reactant R that is capable of interacting with a solute S that passes through the bed, **characterized** in that at least one (**101;201a;301a,a'**) of the flow paths (**101;201a,b;301a,a',b**) comprises/comprise a second porous bed II (**105,205,305**) that is placed upstream of porous bed I (**104,204,304**) and is dummy with respect to interaction with solute S but capable of interacting with a substance DS that is present in a liquid aliquot together with solute S and is capable of disturbing the result of the interaction between solute S and said immobilized reactant R.

2. The microfluidic device of claim 1, **characterized** in that porous bed I (**104,204,304**) and porous bed II (**105,205,305**) are physically separated from each other.

3. The microfluidic device of claim 1, **characterized** in that the upstream end of porous bed I (**104,204,304**) is abutted to the downstream end of porous bed II (**105,205,305**).

4. The microfluidifc device of claim 3, **characterized** in that there is a porous membrane (**106**) between said upstream end and said downstream end.

5. The microfluidic device according to any of claims 1-4, **characterized** in that at least one of porous bed I (**104,204,304**) and porous bed II (**105,205,305**) bed is a packed bed of particles and the remaining porous bed, if any, is a porous monolithic plug.

6. The microfluidic device according to any of claims 1-5, **characterized** in that at least one of porous bed I (**104,204,304**) and porous bed II (**105,205,305**) comprises a solid phase material that is a size exclusion material.

7. The microfluidic device according to any of claims 1-6, **characterized** in that a) the disturbing substance is smaller than solute S and that at least porous bed II (**105,205,305**) in at least one of said at least one flow path comprises a solid phase material that is a size exclusion material having an exclusion limit delaying the disturbing substance from passing through porous bed II) in relation to solutes.

8. The microfluidic device according to any of claims 1-6, **characterized** in that at least one, two or more **(201b;301b)** of the remaining ones of said one, two or more flow paths **(101;201a,b;301a,a',b)** is/are devoid of porous bed II.

9. The microfluidic device according to any of claims 1-7, **characteerized** in that porous bed II in said at least one, two or more flow paths comprises/comprise an immobilised reagent R_{DS} that is capable of interacting with the disturbing substance that is present together with a solutes.

10. The microfluidic device of claims 1-8, **characterised** in that said at least one flow path is two or more flow paths and that R_{DS} in at least one of said two or more flow paths differs from R_{DS} in at least one of the remaining ones of said two flow paths.

11. A microfluidic process carried out in a flow path **(101;201a;301a,a')** of a microchannel structure of a microfluidic device and comprising transporting a liquid aliquot containing a solute S through a porous bed I **(104,204,304)** that is placed in said flow path **(101;201a;301a,a')** and exhibits an immobilized reactant R that is capable of interacting with solute S during the transport, **characterized** in comprising the steps of

(i) providing said flow path **(101;201a;301a,a')** in a form that comprises a porous bed II **(105,205,305)** that is upstream of porous bed I **(104,204,304)** and dummy with respect to interaction with solute S but capable of interacting with a disturbing substance DS,

(ii) providing a liquid aliquot containing said solute S and said disturbing substance in said flow path **(101;201a;301a,a')** in a position that is upstream of porous bed II **(105,205,305)**,

(iii) transporting the aliquot through porous bed II **(105,205,305)**, and

(iv) transporting subsequently solute S through porous bed I **(104,204,304)** to allow for the interaction with reactant R.

12. A microfluidic device in which there is microchannel structure that comprises one, two or more flow paths **(101;201a,b;301a,a',b)** each of which comprises a porous bed I **(104,204,304)** that is common for all of said flow paths and at least one of which **(101;201a;301a,a')** comprises a porous bed II **(105,205,305)** which is upstream of porous

bed I (**104,204,304**), **characterized** in that one or both of porous bed I (**104,204,304**) and porous bed II (**105,205,305**) in said at least one flow path (**101;201a;301a,a'**) comprises a solid phase material containing a generic ligand.3.

13. The microfluidic device of claim 12, **characterized** in the generic ligand in porous bed II (**105,205,305**) in one or more of said at least one flow path (**101;201a;301a,a'**) are the same as in porous bed I.

14. The microfluidic device of claim 12, **characterized** in the generic ligand in porous bed II (**105,205,305**) in one or more of said at least one flow path (**101;201a;301a,a'**) is an affinity counterpart (anti-ligand) to the ligand in porous bed I (**104,204,304**).

15. The microfluidic device of any of claims 12-13, **characterized** in that said ligand is selected amongst biotin and anti-biotins.

16. The microfluidic device of any of claims 12-15, **characterized** in that there is only one flow path (**101**) comprising both porous bed I (**104,204,304**) and porous bed II (**105,205,305**).

17. The microfluidic device of claim 16, **characterized** in that the downstream end of porous bed II (**105,205,305**) is abutted to the upstream end of porous bed I (**104,204,304**), possibly with a porous membrane between the ends.

Fig. 1a

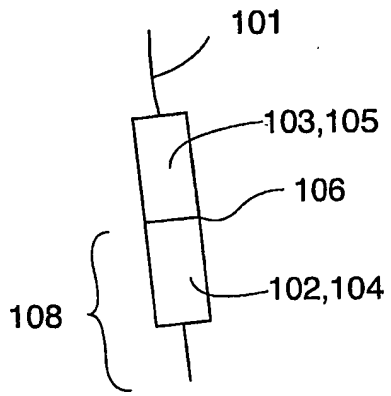


Fig. 1b

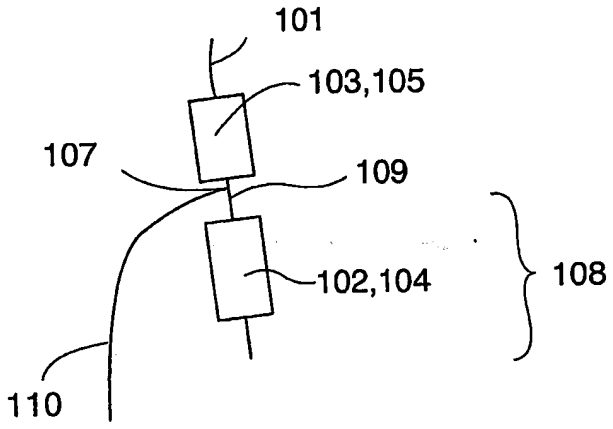


Fig. 2

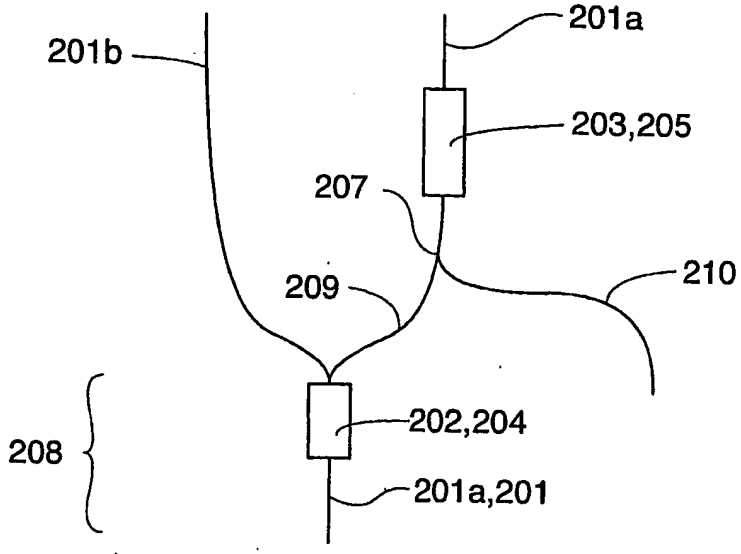

201b
201a
203,205
207
209
210
208
202,204
201a,201

Fig. 3

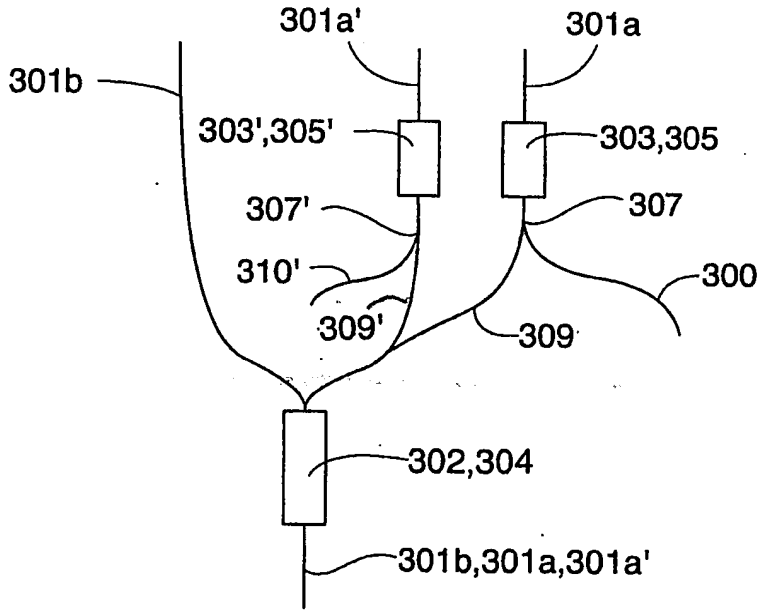

301a'
301a
301b
303',305'
303,305
307'
307
310'
300
309'
309
302,304
301b,301a,301a'

Figure 4

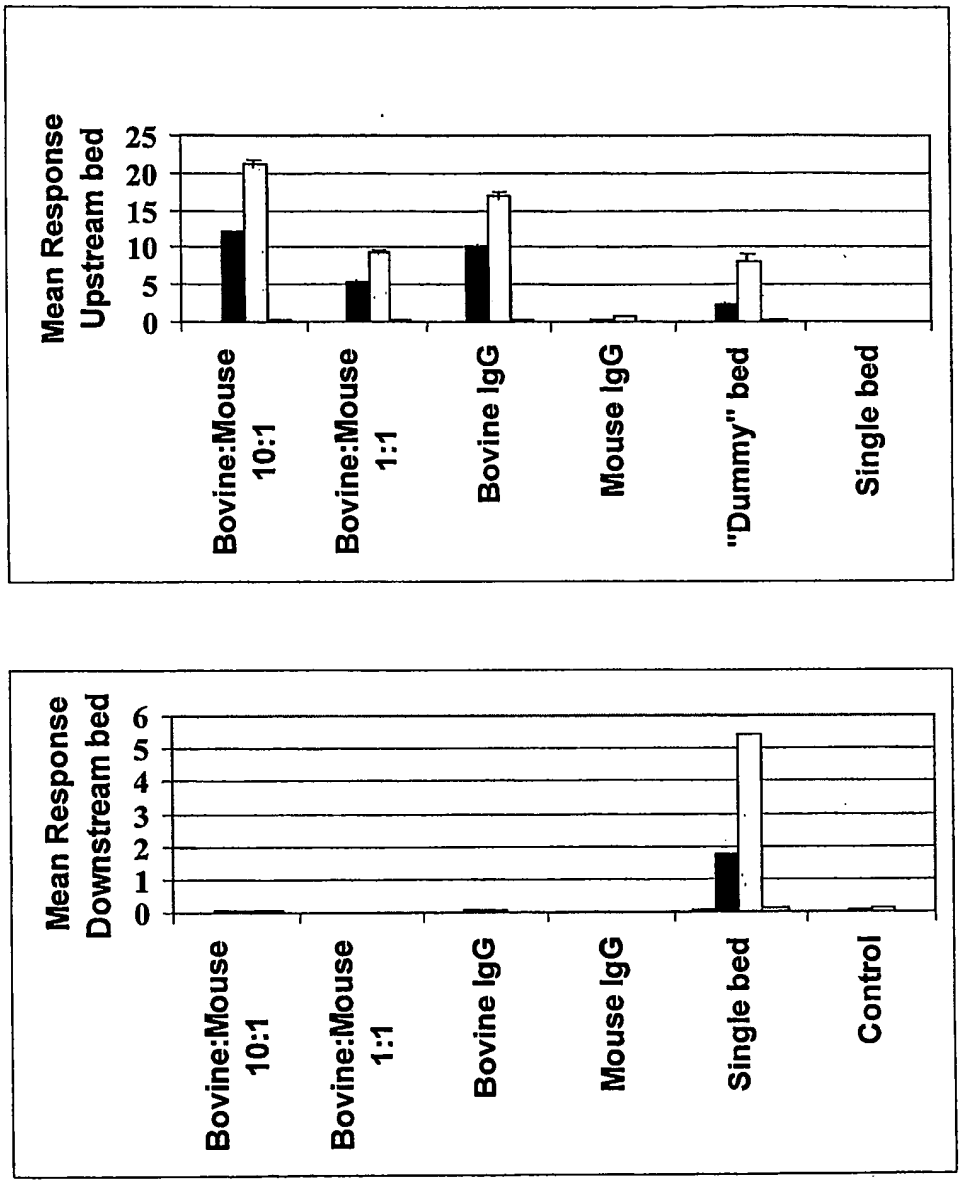

Mean Response Upstream bed
25
20
15
10
5
0
Bovine:Mouse 10:1
Bovine:Mouse 1:1
Bovine IgG
Mouse IgG
"Dummy" bed
Single bed
Mean Response Downstream bed
6
5
4
3
2
1
0
Bovine:Mouse 10:1
Bovine:Mouse 1:1
Bovine IgG
Mouse IgG
Single bed
Control

Figure 5a

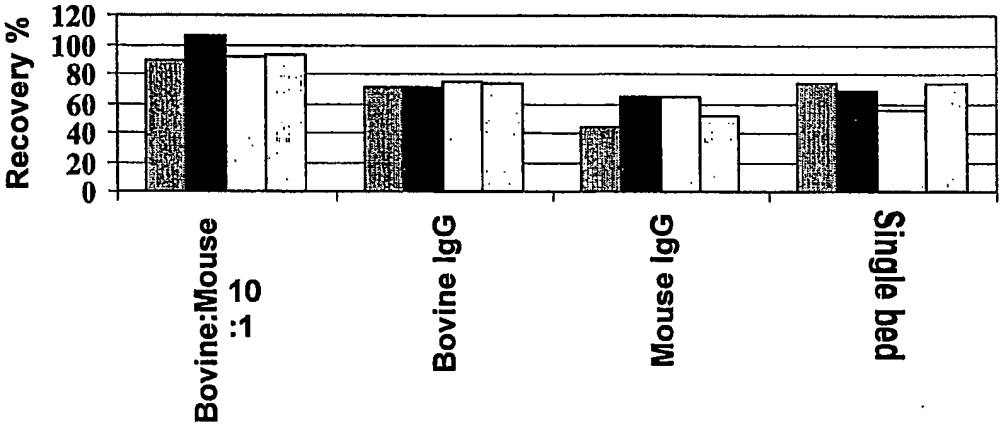
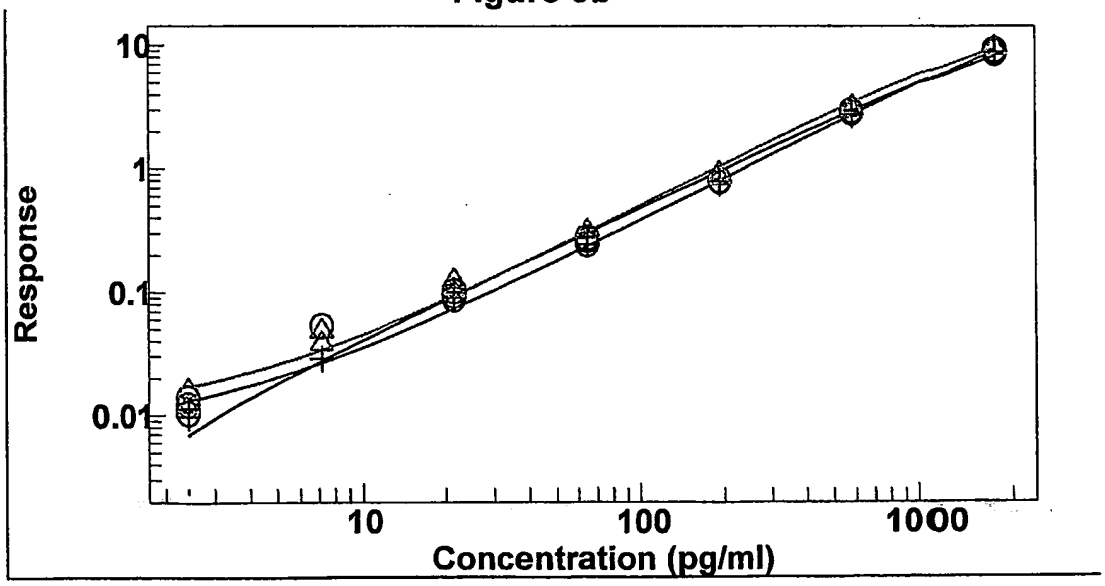


Figure 5b



INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 2005/000114

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: B01L 3/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, WPI DATA, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6632655 B1 (MEHTA, T B ET AL), 14 October 2003 (14.10.2003), abstract ---	1-17
A	WO 02075775 A1 (GYROS AB), 26 Sept 2002 (26.09.2002), abstract ---	1-17
A	US 6479299 B1 (PARCE, J W), 12 November 2002 (12.11.2002), abstract ---	1-17
A	WO 02075312 A1 (GYROS AB), 26 Sept 2002 (26.09.2002), abstract --- --------	1-17

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
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"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 May 2005	11-05-2005
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Åsa Malm /LR Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT
Information on patent family members

01/04/2005

International application No.
PCT/SE 2005/000114

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(54) Title: HYDROPHILIC/HYDROPHOBIC SURFACES

(57) **Abstract:** A microfluidic device that comprises one or a plurality of microchannel structures each of which comprises a microconduit for transport and/or processing of liquid, the inner surface of which comprises a hydrophilic liquid contact surface area (surface area 1) that is delineated in at least one direction by a boundary to a hydrophobic surface area (surface area 2). The characteristic feature is that surface area 2 comprises a rough part that stretches along the boundary.

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HYDROPHILIC/HYDROPHOBIC SURFACES

Technical Field

The present invention relates to a surface that comprises a hydrophilic part delineated in at least one direction by a boundary to a hydrophobic part. The hydrophilic part is intended for retaining or withholding a liquid from spreading into the hydrophobic part and the hydrophobic part for directing liquid into the hydrophilic part. The hydrophilic part will alternatively be called "hydrophilic liquid contact surface" or "surface area 1", and the hydrophobic part for "hydrophobic surface area" or "surface area 2".

This combination of hydrophilic and hydrophobic surfaces is according to the present invention primarily associated with a microchannel structure of a microfluidic device, either completely on inner walls of the microchannel structure or with the hydrophobic surface area being associated with an outer surface that at least partly surrounds an opening of the microchannel structure (to ambient atmosphere).

The surface tension of the liquids concerned is $\geq$ 5mN/m, preferably $\geq$ 10mN/m or $\geq$ 20mN/m and are primarily aqueous.

The terms hydrophilic (wettable) and hydrophobic (non-wettable) contemplate that a surface has a water contact angle $\leq 90°$ or $\geq 90°$, respectively. Hydrophobic compounds or agents are substances that when applied as a coating on a smooth surface gives the surface a water contact angle $\geq 90°$, such $\geq 100°$ or $\geq 110°$. Typically this kind of substances is insoluble in water and may be polymeric or non-polymeric.

Background Technology

Boundaries between hydrophilic and hydrophobic surfaces have previously been utilized in applications where a liquid is to be retained on the hydrophilic side of the boundary for shorter or longer periods of time. In microfluidics this kind of boundary has been used in fluidic functions such as passive valves, anti-wicking functions, vents, liquid-directing functions etc. See for instance: WO 9958245, WO 0185602, WO 02074438, WO 03018198, and WO 03024598 (all of Gyros AB); US 6,926,020, US 6,591,852, US 6,601,613 and US 6,637,463 (all of Biomicro); WO 0190614 (Micronics); WO 9917093

(University of Michigan); US 4,676,274 (Brown); WO 0187486 (Gamera/Tecan); WO 0241996, WO 0242650, and WO 0241995 (all of Pyrosequencing AB); etc.

Hydrophilic surfaces in the form of spots and delineated by boundaries to hydrophobic parts have also been used to collect and/or concentrate aliquots of aqueous liquids to the hydrophilic spots. See for instance US 6,287,872 (Bruker Daltonik GmbH) and WO 9815356 (Molecular Drives Ltd). Hydrophobic spots on a hydrophilic surface have been described in EP 1053784 (Norhoff et al).

A "dispensation" plate comprising on one side larger hydrophilic spots/wells for storing of liquid and on the opposite side smaller hydrophilic spots/wells and a transport capillary between each pair of large and small spot/well. Each hydrophilic spot/well is surrounded by a hydrophobic surface that may be rough. See WO 0107161 (Merck & Co).

Boundaries between hydrophilic and hydrophobic surface areas have been combined with a change in geometric surface characteristics. See the anti-wicking functions described as unit 7 in WO 02074438 (Gyros AB) and the "wells" described in WO 9815356 (Molecular Drives Ltd).

When transporting liquid aliquots through a microchannel structure comprising boundaries of the type discussed above the liquid typically will pass the hydrophobic surface area (surface area 2). Many of the liquids used contain dissolved components that will have a tendency to adsorb to hydrophobic surfaces, for instance surface active components such as detergents and components that exhibit peptide structure (e.g. oligo/polypeptides including proteins). This may be harmful for a fluidic function that is defined by a boundary between a hydrophilic and a hydrophobic surface. The risk for failures in a microchannel structure will increase with its number of surface areas 2/boundaries. The function of valves, vents, anti-wicking functions etc may be disturbed, and not function properly when contacted with liquid a second time, a third etc time etc. Undesired wicking, leakage and spreading of liquid may occur.

Rough/grainy hydrophobic surfaces have been proposed to be particularly suitable for the immobilization of bioaffinity reagents (beads, microtitre wells, fibres tubes etc) (US 5,424,219 (Cytech Biomedical).

Objects

The primary object of the invention is to provide microfluidic devices comprising

a) hydrophilic liquid contact surfaces that have an improved capability of retaining or withholding a liquid of the type discussed above, and

b) hydrophobic surfaces that have an improved capability of directing liquids of the type discussed above into hydrophilic liquid contact surfaces.

The liquid concerned may be aqueous and/or have a selected surface tension ≤ 30 mN/m or ≤ 25 mN/m, such as in the interval from 10 mN/m and upwards.

Another object is to provide microfluidic devices comprising passive valves, anti-wicking functions, vents, liquid-directing functions etc that are based on a boundary between a hydrophilic and hydrophobic surface and that have an improved function, e.g. by permitting repetitive contact with liquids of the kind discussed above.

The invention

The present inventors have recognized that these objects can be accomplished in the case that the hydrophobic part comprises a rough part, i.e. a part with a rough surface that stretches essentially along the boundary on the hydrophobic side.

The invention will have particular advantages for protocols which comprise that at least two liquid aliquot (for instance aliquot I before aliquot II that is before aliquot III etc) is passing the same surface area 2 of a fluidic function of a microchannel structure, for instance with a preceding aliquot that contains harmful substances as discussed above. For this kind of protocols the invention implies that it will be easier to carry out large numbers of parallel runs of essentially the same protocol in the same microfluidic device with a low number of failures, for instance in ≥ 5 microchannel structures, such as ≥ 10 or ≥ 15 or ≥ 25 microchannel structures, typically with no failure of the type discussed above for typical sets of parallel runs. The advantages will become more apparent for increasing number of local surface areas 2 and/or of boundaries per microchannel structure or per flow path that is common for transportation of the aliquots. Thus the largest advantages will be at hand for a microchannel structure/common flow path that comprises two three, four, five, six or more local surface areas 2 and/or of boundaries per microchannel structure/common flow

path. The same also applies to the fluidic functions discussed herein. These hydrophobic areas (surface areas 2) may be different and parts iof different fluidic functions.

The rough part or zone is preferably directly associated with the boundary but there are variants in which there is a hydrophobic zone with a smooth finish between the boundary and the rough part. The hydrophilic part may also comprise a rough part.

A smooth surface is a surface that is not rough.

The roughness may have been obtained from a smooth surface in two major ways: 1) additive roughening and 2) destructive roughening. Both ways may encompass so-called mechanical and/or chemical roughening. Roughness may also be introduced when a surface is initially formed, for instance by moulding, embossing, cutting etc.

Mechanical additive roughening typically comprises that particles are randomly distributed and adhered to a surface. The particles used typically correspond to a population of particles having a mean diameter ≤ 15 μm, such as ≤ 10 μm or ≤ 5 μm or ≤ 1 μm and ≥ 0.01 μm, such as ≥ 0.1 μm or ≥ 0.5 μm or ≥ 1 μm. In the case the boundary is located at an enclosed microcavity/microchannel, the upper limit for the mean diameters is typically ≤ 10 %, such as ≤ 5 %, of the largest cross-sectional dimension (width or depth) at the rough surface in the microcavity/microchannel. These particle sizes refer to the particles as they appear on the hydrophobic surface, e.g. to particle agglomerates if the particles tend to adhere to each other. The particles are typically in the shape of spheres or spheroids, i.e. beaded. Alternatively the particles may have irregular forms. In the case of irregular forms and spheroids the diameters above refer to the "hydrodynamic" diameter.

The particles may expose a hydrophilic or a hydrophobic surface and be porous or non-porous and/or comprise none, one, two or more enclosed holes (hollow particles) etc. In the case the particles are applied in dispersed form to the surface there may be advantages in combining liquid properties with density and/or the size of the particles such that the particles are maintained suspended during application to the surface. Typical particle material includes a) inorganic material such as glass, e.g. borosilicate glass, silica, metal, metal oxide, graphite etc, and b) organic material, such as organic polymers based on

monomers comprising polymerisable unsaturation and/or other groups that permit polymerisation, for instance polymerisable functional groups comprising heteroatoms selected amongst oxygen, sulphur and nitrogen that may or may not participate in the formation of so-called condensation polymers or addition polymers. Inorganic material may also be polymeric.

There are a number of ways to adhere particles to a surface. The particle as such and/or the surface may be self-adhering to each other and/or pretreated with an adherence-promoting agent. This agent may be an adhesive or it may be a solvent partially dissolving an outer layer of the surface or the particles. Alternatively, particles may be distributed on the surface together with a suitable adherence-promoting agent. Moreover, non-sticky particles may be applied to the surface followed by deposition of an adherence-promoting coating.

The application of the particles and the adherence-promoting agent to a surface is typically by printing, spraying, painting and the like.

In a preferred variant the particles are distributed on the surface in dispersed form together with an adherence-promoting agent dissolved or dispersed in the liquid phase of the dispersion. The adherence-promoting agent in this variant is typically a polymer, but also non-polymeric compounds may be used provided they are able to promote adherence. Suitable polymers may be found amongst polymers that are based on monomers comprising polymerisable unsaturation and/or other groups that permit polymerisation, for instance polymerisable functional groups comprising heteroatoms selected amongst oxygen, sulphur and nitrogen that may or may not participate in the formation of so-called condensation polymers or addition polymers. The adherence-promoting agent may be hydrophilic or hydrophobic.

Additive chemical roughening comprises that a chemical or physical reaction is carried out on a surface or in the proximity of the surface, leading to deposition of material on the surface, for instance as precipitates/crystals.

Destructive mechanical roughening comprises methods such as grinding, blasting, etc.

In destructive chemical roughening the surface is degraded in local spots to create small wells, indentation, protrusions and the like. This kind of roughening may be carried out by etching, irradiation etc.

The irregularities in depth/height in rough surfaces obtained by other routes than additive mechanical roughening are typically within the same ranges as for additive mechanical roughening.

After the roughening process the surface may be provided with a surface coating of the desired hydrophobicity. This may in particular be important if the starting surface, the particles, an adhesive, and/or a used adherence-promoting agent is hydrophilic. The method and agents used for introducing a hydrophobic coating at this stage follow the same principles as is well-known in the field of coating. Typically the roughened surface is then coated with a hydrophobic compound, for instance a fluorinated hydrocarbon, paraffin and the like. The preferred surface coatings are typically hydrophobic and in the form of polymers or copolymers that may or may not have been cross-linked, for instance based on ethylene, propylene, butadiene, fluorinated alkenes, etc. Suitable hydrophobic polymers can be found amongst polymers that are based on monomers comprising polymerisable unsaturation and/or other groups that permit polymerisation, for instance polymerisable functional groups comprising heteroatoms selected amongst oxygen, sulphur and nitrogen that may or may not participate in the formation of so-called condensation polymers or addition polymers. See the experimental part, US 6,447,919 (Cytonix), and SE 0400917, corresponding US provisional application (filed on the same day as SE 0400917) and the corresponding International Patent Application (all three Gyros AB).

Roughness may be expressed as arithmetric average roughness (R_a), which is also known as arithmetic average (AA), centre line average (CLA), and arithmetical mean deviation of the profile. This kind of roughness corresponds to the area between the roughness profile and its mean line, or the integral of the absolute value of the roughness profile height over the evaluation length:

$$R_a = \frac{1}{L}\int_0^L |r(x)|\,\mathrm{d}x$$

When evaluated from digital data, the integral is normally approximated by a trapezoidal rule:

$$R_a = \frac{1}{N}\sum_{n=1}^{N}|r_n|$$

Graphically, the average roughness is the area between the roughness profile and its centre line divided by the evaluation length (normally five sample lengths with each sample length equal to one cutoff).

In the formula above L is evaluation length, r(x) is roughness at position x, N is total number of values and r_n is roughness at pixel n.

Effective roughness R_a for the invention is typically found within the intervals of 0.01-15 μm, such as 0.1-15 μm or 0.5-10 μm.

The optimal interval of the depths and heights of the indentations and/or projections/protrusions, respectively, in the rough surface depends on a) the liquid to be used, for instance its surface tension, b) whether or not the surface is part of an enclosed microconduit/microcavity and the dimensions of such a microconduit/ microcavity etc. Typically experimental testing is required for optimization. The same also applies for R_a-values.

In many variants of the invention, application of the present innovative principle means that the water contact angle macroscopically is increased, e.g. with ≥ 2°, such as ≥ 4° or ≥ 5° or ≥ 10°, compared to the hydrophobic zone without the roughening.

In preferred variants the water contact angle of surface area 1 is selected in the interval ≤ 60° such as ≤ 50° or ≤ 40° or ≤ 30° or ≤ 20°. Surface area 2 typically has a water contact angle ≥ 100°, such as ≥ 110° or ≥ 125° or ≥ 135°, and may be superhydrophobic, i.e. a water contact angle above 150°.

The intervals for water contact angles refer to static water contact angles and advancing water contact angles. Advancing angles typically are higher than static angles.

Contact angles refer to values at the temperature of use, typically +21°C, are static or advancing. Static angles are measured by the method given in WO 0056808 (Gyros AB). Advancing angles are measured as given in the experimental part.

The innovative principle is applicable to surfaces made of different kinds of material. Typical material includes a) inorganic material such as glass, e.g. borosilicate glass, silica, metal, metal oxide, etc, and b) organic material, such as organic polymers (plastic material) based on monomers comprising polymerisable unsaturation and/or other groups that permit polymerisation, for instance polymerisable functional groups comprising heteroatoms selected amongst oxygen, sulphur and nitrogen.

The hydrophilic liquid contact surface, the hydrophobic surface and the boundary are typically present as a part of a passive valve, an anti-wicking function, a vent, a liquid-directing function and the like in an enclosed microchannel structure of a microfluidic device. The hydrophobic surface area of certain liquid-directing functions may be present on the outer surface of a microfluidic device as discussed above under the heading "Technical Field".

Passive valves, anti-wicking functions and vents that utilize the innovative principle are typically present in microconduit parts of microchannel structures in which the intersections of inner side-walls define one, two, three, four or more length-going edges. See for instance figure 1 of WO 02074438 (Gyros AB). The boundary and/or rough part/zone of the hydrophobic part/zone (surface area 2) typically stretch between two edges of one, two or more of the inner sidewalls (= top wall, bottom wall and walls between top and bottom walls), preferably at least in opposing sidewalls and/or preferably starting in the edges of an inner wall concerned. In other words for each of the functions, one, two, three or more inner sidewalls may comprise the boundary and a surface area 1 and a surface area 2 that defines the boundary. The direction of the boundary is preferably essentially perpendicular to the flow direction, i.e. 90° ± 45°.

In a passive valve that is based on the innovative principle the hydrophobic part (surface area 2) comprising the rough part is typically present as a hydrophobic zone in one, two,

three, four or more of the inner side-walls at the position of the valve. The position of the hydrophobic zone in the flow direction may coincide with the position of a local change in geometric surface characteristics, although they may be present in different sidewalls. The hydrophobic zones in opposing sidewalls should be at essentially the same position along the flow path/microconduit. This kind of valve is typically present at outlet ends of volume-defining microcavities, reaction microcavities, outlet ends of microconduits etc, i.e. the same positions as for conventional passive valves that are based on a boundary between a hydrophilic and a hydrophobic surface area. This means that the rough part may be present in the hydrophobic surfaces 205a,b in figure 2; 321a, b, c,d, e; 322, 323, 335 in figures 3; 408,423 in figures 4; 607,608,610 in figure 6; 809 in figure 8; 1007 in figure 10; 1206,1208 in figure 12; and 1310,1313 in figure 13a of WO 02074438 (Gyros AB).

In an anti-wicking function that is based on the innovative principle the hydrophobic surface area comprising the rough part typically is present as a hydrophobic zone in one, two, three, four or more inner sidewalls. The hydrophobic zones and roughened parts in opposing sidewalls are typically slightly displaced relative to each other (in the flow direction) if a valve function is to be minimized. The position in the flow direction of this kind of zone/hydrophobic part may partly or completely coincide with the position of a local change in geometric surface characteristics, for instance in different inner sidewalls. The boundary and the rough part in an anti-wicking function stretch between edges in the same manner as for valves. Anti-wicking functions are typically present immediately upstream to and/or between volume-metering microcavities that define a distribution manifold, and at other positions where it is important to keep undesired liquid transport by wicking at a minimum. This means that the roughened zone may be present in the hydrophobic surfaces 321g, 314 in figures 3; 426 in figure 4b; 804/805 in figure 8a; 1106/1107 in figure 11b; 1209 in figure 12; and 1312 in figure 13 of WO 02074438 (Gyros AB).

In a vent that is based on the innovative principle, the hydrophobic surface area comprising the rough part may be present as a local hydrophobic zone or stretch from the venting position to the outlet end of the venting microconduit. The roughened zone may thus be present in the hydrophobic surfaces 208 in figure 2; 321,336 in figures 3, and 406 in figure 4 of WO 02074438 (Gyros AB).

Liquid-directing functions that are based on the innovative principle may be present within a microfluidic device, for instance at branchings of microchannel structures or in distribution manifolds between volume-defining microcavities to assist splitting of a larger liquid aliquot into smaller aliquots. This means that the rough part may be present in the hydrophobic surfaces 205a, 208 in figure 2; 321,336 in figure 3; 405, 406, 408 in figure 4; and 610 in figure 6 of WO 02074438 (Gyros AB). Liquid-directing functions according to the innovative principle may also define complementing hydrophilic flow paths in each surface of two planar substrates. When the two surfaces are apposed to each other at a capillary distance with complementary flow paths aligned, a microfluidic device will be formed in which the complementary flow paths will define the microchannel structures of the device. See further WO 9958245 (Gyros AB). The boundary between surface area 1 and surface area 2 may stretch between inner edges in the same manner as for valves, vents and anti-wicking functions in cases where liquid is to be directed into a particular branch at a branching.

Other kinds of liquid-directing functions according to the invention may be present on the outside of a microfluidic device and associated with inlet and/or outlet ports. Liquid-directing functions at an inlet port may assist in guiding liquid into a microchannel structure. Liquid-directing functions at an outlet port may assist in retaining liquid in the port and/or within the device. The roughened part may thus be present in the hydrophobic surfaces 321 of figure 3; 1105 of figure 11; and 1210 of figure 12 of WO 02074438 (Gyros AB).

The boundary between a surface area 1 and a surface area 2 may be part of one, two, three or more functionalities. Thus, a valve or a vent may also have an anti-wicking function and/or liquid-directing function, for instance.

In microconduits of a microfluidic device, the hydrophobic part (surface area 2) and/or only its rough part may extend in the flow direction along a distance that is from 0.1 times to 10, 100, 1000 or more times the width or depth of the microconduit. Comparison is made with the width and depth at the upstream or downstream end of the hydrophobic part.

Boundaries according to the innovative principle may also be used to define an array of hydrophilic spots on a hydrophobic surface or an array of hydrophobic spots on a

hydrophilic surface. Arrays of hydrophilic spots may be used to collect liquid aliquots, for instance in the form of drops, with one aliquot on each spot. In the case the array is open to ambient atmosphere the liquid will evaporate thereby concentrating solutes to the hydrophilic spots surrounded by the roughened surface. This kind of design without roughening has been described in WO 02075775 (Gyros AB) (surfaces 1011 and 1012 of figure 7b). The boundary may be associated with a local change in geometric surface characteristics, for instance to define a well that will improve retaining of an aqueous aliquot as outlined without roughening in WO 02075775 (Gyros AB; 1011 and 1012 of figure 7b). Additional kinds of hydrophobic surfaces to which the innovative principle with roughening may be applied are given in WO 9958245 (Gyros AB).

Microfluidic devices

A microfluidic device contemplates a device that comprises one or more microchannel structures in which liquid flow is used for transporting and processing liquid aliquots containing various kinds of reactants, analytes, products, samples, buffers and/or the like. Processing in this context means operations such as performing chemical and/or biological reactions, synthesizing, isolating, purifying, separating, fractionating, concentrating, diluting, mixing, volume-metering/defining, heating, cooling etc. The mere transporting of a liquid within a microchannel of a device does not qualify the device to be a microfluidic device. Typically at least some kind of fluidic function, such as a valve, needs to be present in the device and used, including also processing of the liquid.

A microconduits is a part of a microchannel structure and may be straight, branched, angled,curved etc. Micrconduits and also microchannel structures in general have in preferred variants intersecting inner sidewalls that define length-going edges as discussed above for different functions. See also figur 1 of WO 02074438 (Gyros AB).

The volumes of the aliquots are typically in the nanolitre (nl) range. A microchannel structure comprises all the functionalities that are necessary for performing an intended experiment within the microfluidic device. Each structure typically contains one or more cavities and/or conduits that have a cross-sectional dimension that is $\leq 10^3$ μm, preferably $\leq 5 \times 10^2$ μm, such as $\leq 10^2$ μm. The nl-range has an upper limit of 5,000 nl. In most cases it relates to volumes ≤ 1,000 nl, such as ≤ 500 nl or ≤ 100 nl.

A microchannel structure thus may comprise one, two, three or more functional parts selected among: a) inlet arrangement comprising for instance an inlet port/inlet opening, possibly together with a volume-metering unit, b) microconduits for liquid transport, c) reaction microcavity; d) mixing microcavity; e) unit for separating particulate matters from liquids (may be present in the inlet arrangement), f) unit for separating dissolved or suspended components in the sample from each other, for instance by capillary electrophoresis, chromatography and the like; g) detection microcavity; h) waste conduit/microcavity; i) valve; j) vent to ambient atmosphere; etc. A functional part may have more than one functionality, e.g. reaction microcavity and a detection microcavity may coincide. Various kinds of functional units in microfluidic devices have been described by Gyros AB/Amersham Pharmacia Biotech AB: WO 9955827, WO 9958245, WO 02074438, WO 0275312, WO 03018198, WO 03024598 and by Tecan/Gamera Biosciences: WO 0187487, WO 0187486, WO 0079285, WO 0078455, WO 0069560, WO 9807019, WO 9853311.

The microfluidic device may also comprise common microchannels/micro conduits connecting different microchannel structures. Common channels including their various parts such as inlet ports, outlet ports, vents, *etc.*, are considered part of each of the microchannel structures they are communicating with.

Common microchannels make it possible to construe microfluidic devices in which the microchannel structures form networks. See for instance US 6,479,299 (Caliper)

Each microchannel structure has at least one inlet opening for liquids and at least one outlet opening for excess of air (vents) that in certain variants also may be used for outlet of liquids.

The number of microchannel structures/device is typically ≥ 10, e.g. ≥ 25 or ≥ 90 or ≥ 180 or ≥ 270 or ≥ 360.

Different principles may be utilized for transporting the liquid within the microfluidic device/microchannel structures between two or more of the functional parts described

above. Inertia force may be used, for instance by spinning the disc as discussed in the subsequent paragraph. Other useful forces are capillary forces, electrokinetic forces, non-electrokinetic forces such as capillary forces, hydrostatic pressure *etc.*

The microfluidic device typically is in the form of a disc. The preferred formats have an axis of symmetry (C_n) that is perpendicular to the disc plane, where n is an integer $\geq 2, 3, 4$ or 5, preferably ∞ (C_∞). In other words the disc may be rectangular, such as in the form of a square, or have other polygonal forms. It may also be circular. Once the proper disc format has been selected centrifugal force may be used for driving liquid flow, e.g. by spinning the device around a spin axis that typically is perpendicular or parallel to the disc plane. In the most obvious variants at the priority date, the spin axis coincides with the above-mentioned axis of symmetry.

For preferred centrifugal-based variants, each microchannel structure comprises an upstream section that is at a shorter radial distance than a downstream section relative to the spin axis.

The preferred devices are typically disc-shaped with sizes and forms similar to the conventional CD-format, e.g. sizes that corresponds CD-radii that are the interval 10% - 300 % of the conventional CD-radii. The upper and/or lower sides of the disc may or may not be planar.

Microchannels/microcavities of a microfluidic device may be manufactured from an essentially planar substrate surface that exhibits the channels/cavities in uncovered form that in a subsequent step are covered by another essentially planar substrate (lid). See WO 9116966 (Pharmacia Biotech AB) and WO 0154810 (Gyros AB). The material of the substrates may be selected as discussed above.

EXPERIMENTAL PART

EXAMPLE 1. Preparation of innovative surfaces

0.4-2.0% (w/w) Aerosil™ R972 methylated silica colloids (DeGussa, d = 11 nm) were added to a 0.05% solution of Teflon-AF™ 2400 (DuPont Polymers, DE, USA). The mixture was applied by spraying or dipping onto Zeonor™ 1420R (Zeon Corp., Japan) which had been surface treated with an oxygen plasma (Plasma Electronic, Germany). The resulting surfaces had advancing / receding water contact angles of 165-170° / 130-170 °.

2% (w/w) Aerosil™ R972 was added to PFC602A (Cytonix Corp., MD, USA), which is a 2% solution of polyperfluorooctalmethacrylate in HFE-7100 (3M Belgium N.V.). Sprayed or dipped surfaces had advancing / receding water contact angles of 169-174° / ~165°.

The adhesion of these coatings to oxygen plasma-treated Zeonor™ could be greatly improved by mixing, for example 2% PFC602A in a 1:1 ratio with perfluorodecylmethacrylate and 0.1-0.4% Esacure™ TZT (Lamberti, Italy) and 1% Aerosil™ R972. The mixture required the addition of acetone (10%) in order to dissolve Esacure™ TZT. After drying, the coating was cured by illumination under a UV-lamp for 2 minutes (500 W, Efsen, Denmark). The resulting coating was wash stable to 95% ethanol, and had advancing / receding water contact angles of ~175°/135°.

Measurement of Contact Angles

Water contact angles were measured using a Ram é-Hart goniometer. Advancing contact angles were determined by increasing the drop volume until the contact line just started to move. The receding contact angle was determined in a similar way upon decreasing the drop volume.

EXAMPLE 2. BEHAVIOR OF LIQUIDS CONTAINING SURFACE-ACTIVE COMPONENTS ON THE INNOVATIVE SURFACES

In many applications of microfluidic devices, hydrophobic surfaces in valves and in anti-wicking functions are subject to repeated contact with liquids containing surface-active components. These surface-active components may then adsorb to the hydrophobic surface, which in many cases gives rise to functional failure of valves and/or anti-wicking functions. In order to study if this problem could be overcome by the inventive concept a number of combinations of particles and hydrophobizing agents were tested for contact

with liquids containing BSA (bovine serum albumin), serum, Tween etc. In some experiments short as well as prolonged contacts were compared. The effect was studied as advancing contact angles and compared with the surface tension of each solution. Table 1 gives representative results for a deposition solution containing Teflon AF (0.05% in FC-75) and dispersed 5 μm particles (2% Chromasil-NH_2, Eka Nobel, Sweden). The solution was sprayed onto a surface of Zeonor and dried before subsequent measurements.

Table 1. Contact angles and surface tensions of different solutions containing surface active agents. Plastics: Zeonor.

Solution	Advancing contact angle/smooth surface	Advancing contact angle/rough surface	Surface tension
Water	135	165	77
1% BSA in PBS/short contact	125	158	52
1% BSA in PBS/prolonged contact (90 s)	123	140	52
serum	#	#	50
Tween 0.1%	96	106	37*

see text

*Surface tension extrapolated from values measured for Tween 20 concentrations of 0.012% (39 $mJ.m^{-2}$) and 1.2% (35 $mJ.m^{-2}$). Note: even the lowest concentration is higher than the critical micelle concentration (cmc = 0.0072%).

The rough surface is according to the invention and the smooth surface according to the prior art.

This results show that the innovative surface remains superhydrophobic on contact with 1 % BSA, the advancing contact angle decreasing somewhat after a longer contact with 1 % BSA. While the advancing contact angle was not measured directly for serum, the small

difference in surface tension between 1 % BSA and serum would suggest that enhanced hydrophobicity is maintained even for the latter. Surface tension of Tween 20 solutions is considerably lower, so that contact angles on both smooth and rough Teflon AF surfaces is considerably reduced. The difference in advancing contact angles between rough and smooth surfaces is also reduced.

Certain innovative aspects of the invention are defined in more detail in the appending claims. Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined by the appended claims. Moreover, the scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, composition of matter, means, methods and steps described in the specification. As one of ordinary skill in the art will readily appreciate from the disclosure of the present invention, processes, machines, manufacture, compositions of matter, means, methods, or steps, presently existing or later to be developed that perform substantially the same function or achieve substantially the same result as the corresponding embodiments described herein may be utilized according to the present invention. Accordingly, the appended claims are intended to include within their scope such processes, machines, manufacture, compositions of matter, means, methods, or steps.

CLAIMS

1. A microfluidic device comprising one or a plurality of microchannel structures each of which comprises a microconduit for transport and/or processing of liquid, the inner surface of which comprises a hydrophilic liquid contact surface area (surface area 1) that is delineated in at least one direction by a boundary to a hydrophobic surface area (surface area 2), **characterized** in that surface area 2 comprises a rough part that stretches along the boundary.

2. The microfluidic device of claim 1, **characterized** in that the roughness of said rough part has been introduced by a method, which comprises additive or destructive roughening.

3. The microfluidic device of claim 2, **characterized** in that said roughening comprises additive roughening by deposition of particles, for instance of particles having mean diameter of ≤ 15 μm, such as ≤ 10 μm or ≤ 5 μm.

4. The microfluidic device of claim 3, **characterized** in that said particles have been deposited in dispersed form, e.g. added by spraying or printing, and that the liquid phase of the dispersion preferably comprises an agent promoting adherence of the particles to surface area 2.

5. The microfluidic device of claim 3, **characterized** in that the particles have been deposited in dry form subsequent to pre-treatment of surface area 2 to allow for adherence of the particles.

6. The microfluidic device of any of claims 3-4, **characterized** in that the roughness has been introduced by additive roughening comprising applying the particles together with a hydrophobic adherence-promoting agent and/or hydrophobizing the rough part including the particles after the application of the particles.

7. The microfluidic device of any of claims 2-6, **characterized** in that the the roughness has been introduced by destructive roughening, e.g. chemical and/or mechanical destructive roughening.

8. The microfluidic device of any of claims 1-7, **characterized** in that the rough part exposes a hydrophobic polymer on its surface.

9. The microfluidic device of claims 1-8, **characterized** in that surface area 1, the boundary and surface area 2 are defined on a substrate comprising plastic material, typically polymeric at least in the surface, or of inorganic material such as glass, silica, metal including metal alloy, metal oxide etc.

10. The microfluidic device of any of claims 1-9, **characterized** in that said microconduit has two, three or more inner sidewalls, which pair-wise intersect each other to define one, two or more inner edges which each extends along said at least one microconduit, and said boundary extends between two edges in at least one of said inner sidewalls..

11. The microfluidic device of claim 10, **characterized** in that said boundary extends in a direction essentially perpendicular to the transport direction in said microconduit.

12. The microfluidic device of any of claims 10-11, **characterized** in that the boundary is part of a fluidic functionality selected amongst passive valves, anti-wicking functions, inlet or outlet vent to ambient atmosphere, and liquid-directing functions.

13. The microfluidic device of any of claims 1-9, **characterized** in that

a) surface area 1 is a spot (hydrophilic spot 1) completely delineated by said boundary and present on an array surface comprising an array of hydrophilic spots of essentially the same size and form as hydrophilic spot 1,

b) the array surface is present on one, two or more inner sidewalls of a microcavity that is part of said microconduit, and

c) the distance between an inner wall, which comprises said array surface, and the opposing inner sidewall is ≤ 2000 μm, such as ≤ 1000 μm or ≤ 500 μm or ≤ 300 μm.

14. The microfluidic device of any of claims 1-13, characterized in that surface area 2 is located on the outside of the device in association with an opening of the microconduit and partially or completely surrounds the opening.

15. The microfluidic device of any of claims 1-14, **characterized** in that each of said microchannel structures comprises two, three, four, five or more of said surface area 2.

16. The microfluidic device of any of claims 1-14, **characterized** in that each of said microchannel structures comprises one, two, three or more passive valve functions and/or one, two, three or more anti-wicking functions each of which function comprises said boundary.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 2004/000794

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: B81B 1/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C03C, B01C, B81B, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL,WPI,PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 0107161 A1 (MERCK & CO,INC.), 1 February 2001 (01.02.2001), page 15, line 6 - line 15, abstract --	1-16
A	US 5424219 A (G.F.JIRIKOWSKI), 13 June 1995 (13.06.1995), column 5, line 21 - line 49 --	1-16
A	WO 0241996 A1 (PYROSEQUENCING AB), 30 May 2002 (30.05.2002), abstract --	1-16
A	GB 1474017 A (G.D.SEARLE CO.LTD.), 18 May 1977 (18.05.1977), claim 1 --	1-16

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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"P" document published prior to the international filing date but later than the priority date claimed
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 August 2004	25 -08- 2004
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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